12-2014

Population Genetics, Distributions and Phenology of Bombus Latreille, 1802 and Xylocopa Latreille, 1802 (Hymenoptera: Apidae)

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Entomology

by

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December 2014
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ABSTRACT

This work addresses multiple knowledge gaps in bee ecology, population health and phylogeography in order to provide insights into the changing distributions of native bees. A comparison of Arkansas bumble bee records mirrors range-wide surveys, with records of stable species (*Bombus bimaculatus* Cresson, 1863 and *B. impatiens* Cresson, 1863) increasing three-fold, and records of the declining *B. pensylvanicus* (DeGeer, 1773) dropping to 60% of historical levels. However, nationally-recommended conservation-genetics tools did not mirror these results on a regional level. Stable and declining species had equivalent genetic diversity in samples from Arkansas and Tennessee (H_S range: 0.46–0.63). Diploid males, which indicate inbreeding, were only detected in the species known to be stable, *B. bimaculatus* and *B. impatiens.* This could be an indication of broad similarity of these taxa in the region, or it could indicate that recommended microsatellite-based tools are less likely to detect genetic signatures of declines at a localized level.

A three-year survey of floral associations and seasonality in a community of eight bee species across Northwest Arkansas found that local and landscape factors had no effect on the differential abundances of this community, but overall abundance increased with increasing plant richness at each site ($F_{(1,11)}$=45.62, p<0.001), as did the abundance of each bumble bee species. Bumble bees with long glossae, a group usually thought to be at higher risk of decline, were more specialized in their flower use, and although their food choices overlapped (O_{12}=0.54), they skirted potential competition by maintaining different phenologies.

Subspecies status was maintained for *Xylocopa virginica texana* Cresson, 1872, but not for *X. v. krombeini* Hurd, 1961. This morphological east-west differentiation is additionally supported by mitochondrial phylogeographic analyses which suggest that *X. virginica* expanded

from multiple glacial refugia. On the other hand, *X. micans* haplotypes are consistent with a single origin, likely west of the Mississippi River. In spite of its interpopulation homogeneity, *X. micans* is quite genetically diverse (H_d=0.91±0.03) compared to *X. virginica* and (H_d=0.78±0.02), consistent with Hewitt's leading-edge hypothesis for range disparity. Together, these results highlight the importance of an ecological perspective in the quest to understand bee distributions and decline.

ACKNOWLEDGEMENTS

I would like to thank a number of people that have aided me in my endeavors, although none of them are to blame for the results. Firstly, I wish to thank my advisor, Allen Szalanski for the unwavering support, encouragement, advice and guidance he has provided throughout the years. I would also like to thank my committee members, Marlis Douglas, Jeffery Barnes, Donn Johnson and Kenneth Kvamme for always being willing to help and offer encouragement, whether my crises were scientific or existential. I extend the deepest gratitude to my companions in the department, Susan Osredker, Soo-Hoon (Sam) Kim, Ace Lynn-Miller, Bryan Petty, Mark Janowiecki and Clinton Trammel whose willingness to join me in adventures and conversations kept me both sane and engaged over the years. I also thank my parents, Lloyd Tripodi and Dawn Hall for their overwhelming enthusiasm and encouragement. Finally, none of this would have been possible without the incredible support of my best friend, Josh Gladwin, without whom I would have forgotten to eat and never would have dreamed of pursuing a Ph.D.

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I. INTRODUCTION

A. BEES AND POLLINATION

Best known for their pollination services, the bees (Hymenoptera: Apoidea: Anthophila) are a large and diverse group of insects with over 17,500 described species worldwide (Michener 2007). Animal pollinators are responsible for pollinating 35% of the food crops directly consumed by humans globally, and the majority of these pollinators are bees (Klein, *et al.* 2007a). In the United States (US), the imported European honey bee, *Apis mellifera,* Linnaeus, 1758 (Hymenoptera: Apidae) is managed for pollination purposes as well as for honey and other products, but unmanaged, native bees also provide agricultural pollination services. The estimated annual value of pollination services provided to United States agriculture by unmanaged, non-*Apis* insect pollinators, including bees, exceeds 3 billion dollars (Losey and Vaughan 2006; Calderone 2012). Like *A. mellifera,* many native bees are polylectic and are capable of pollinating many field crops such as berries (*Rubus* and *Vaccinium*), melons and cucumbers (*Cucumis* and *Citrullus*), canola and cole crops (*Brassica*), orchard fruits (*Malus* and *Prunus*) and squash (*Cucurbita*) (reviewed in Klein, *et al.* 2007b). In addition, bumble bees (*Bombus* Latreille, 1802, (Hymenoptera: Apidae)) and carpenter bees (*Xylocopa* Latreille, 1802, (Hymenoptera: Apidae)) exhibit a sonicating behavior which honey bees do not perform. This behavioral and morphological character is an active form of pollination, in which the bee creates a sonic vibration at a specific frequency by a spasm of the indirect flight muscles (Buchmann, *et al.* 1977). This vibration releases pollen even from recalcitrant, poricidal anthers in which the pollen is held inside the anther, rather than presented on the exterior. Such plants (*e.g.* solanaceous flowers like tomatoes and ericaceous ones like blueberries) require buzz pollinators for sufficient fertilization, and are closely tied to their pollinators even in the absence of species-

specific specialization. Even self-pollinating crops can benefit from sonication by buzz-pollinating bees. For example, tomato fruit set and fruit volume increased by visits from sonicating native bees (Greenleaf and Kremen 2006). *Xylocopa virginica* (Linnaeus, 1771) has a notorious reputation for nectar robbery, a behavior in which the foraging bee uses its galeae to slit open the corolla of a flower. This allows the bee to access the nectar reserves without making direct contact with the sexual portions of the inflorescence, bypassing pollination (Balduf 1962). Although nectar robbery is generally viewed as antithetical to crop pollination, there is evidence that it increases blueberry pollination by *A. mellifera* (Sampson, *et al.* 2004). With recent honey bee declines impacting managed pollination services, maintaining diverse populations of native pollinators is essential to assuring the resilience and sustainability of agricultural systems (Winfree, *et al.* 2007). There are indications, however, that some native bee species, particularly in the genus *Bombus*, might be experiencing declines in the United States (Goulson, *et al.* 2008a; Grixti, *et al.* 2009).

B. A REVIEW OF THE POTENTIAL CAUSES OF BUMBLE BEE DECLINE

Many factors have been correlated with bumble bee decline, but no consistent pattern has emerged. One of the more interesting complications in the search for causes of bee decline is that, although bee decline seems widespread both geographically and phylogenetically, there are some species that seem to be faring quite well, while others are not (*e.g. Bombus* in North America, Cameron, *et al.* 2011). The potential causes of bee decline might be easily divided into two classes: intrinsic and extrinsic factors. We would expect that extrinsic factors, *e.g.* climate change, habitat loss, pesticide use, novel pathogens, etc., would affect all bees similarly, yet we see that some species are seemingly stable while others are notably declining. This suggests that intrinsic, species-level characteristics are important factors to consider, either because they are

the true drivers of bee decline or because they mitigate or exacerbate extrinsically-driven decline in certain species. There are differing opinions on which intrinsic characteristics leave bee species vulnerable, and what extrinsic factors are responsible for driving declines in vulnerable species. The true scenario is likely that bee decline is driven by an interaction between extrinsic factors that are exerting additional environmental pressures on bee species with varying levels of susceptibility due to intrinsic differences among species. Here, I review some of the major hypotheses that have been proposed for the factors driving bumble bee decline worldwide. Many of these are not mutually exclusive and might in fact act synergistically or even mitigate the effects of one another.

Variety is key: Declining species exhibit lower genetic diversity than stable species

This is a classic hypothesis to explain differential fitness among populations that seem ecologically similar, yet exhibit variability in population stability. Genetically depauperate populations might be more vulnerable to undirected genetic drift and might lack the variation to effectively weather exposure to new threats, such as novel pathogens. Wild populations of *B. terrestris* (Linnaeus, 1758) in Sweden show a negative relationship between genetic diversity and infection with the pathogen *Nosema bombi* Fantham and Porter, 1914 (Microsporidia: Nosematidae), with colonies founded by genetically diverse queens having lower rates of infection than their less diverse counterparts (Huth-Schwarz, *et al.* 2012). Within the bumble bee literature, there seems to be general agreement that declining species exhibit lower genetic diversity. Using microsatellite markers, expected heterozygosity levels were lower in North American species that are relatively less abundant than their stable congeners (H_E- declining- *B. occidentalis* Greene, 1858: 0.584 and *B. pensylvanicus* (DeGeer, 1773): 0.577 vs. stable: *B. vosnesenskii* Radoszkowski, 1862: 0.676, *B. bifarius* Cresson, 1878: 0.700, *B. impatiens*

Cresson, 1863: 0.692 and *B. bimaculatus* Cresson, 1863: 0.693) (Cameron, *et al.* 2011). In the United Kingdom, *B. muscorum* (Linnaeus, 1758), a declining species, exhibited greater population structure and lower heterozygosity and allelic richness than a common, stable species *B. jonellus* (Kirby, 1802) (Darvill, *et al.* 2010). The authors attribute this to the greater dispersal capacity of *B. jonellus*, suggesting that this species can respond more quickly to habitat loss than its less fortunate congener, *B. muscorum.* Additionally, isolated populations of *B. muscorum* and *B. sylvarum* (Linnaeus, 1761) have been shown to include diploid males, a notable indication of inbreeding in hymenopterans (Darvill, *et al.* 2006; Ellis, *et al.* 2006).

Most Hymenopterans have haplo-diploid sex determination that includes an additional, complementary sex allele located at a single locus (sl-CSD). If sl-CSD alleles are identical in a diploid offspring, the resulting diploid male is usually sterile, terminated or, worse yet, viable, yet unable to father normal offspring. When producing sperm, meiosis is terminated at the first stage by normal male Hymenopterans, a process likely conserved in diploid males as well (Cowan and Stahlhut 2004). Viable diploid males that mate will father offspring that are triploid and thus inviable or sterile (Zayed 2009). The consequences of inbreeding in haplo-diploid, sl-CSD organisms can be quite severe, particularly for social bees which depend on the production of a worker caste and have a lower effective population sizes than census data might indicate. The special conditions governed by the unusual sex determination mechanisms in bees leave them particularly prone to extirpation, a condition that has been termed the “diploid male vortex” route to extinction (Zayed and Packer 2005). Under normal circumstances, the production of diploids should yield either colony workers or reproductive queens for the next generation, rather than males, which do not contribute to colony growth or found new colonies. Although some Hymenopterans have compensating factors that mitigate the consequences of inbreeding (*e.g.*

functional diploid males of *Euodynerus foraminatus* (de Saussure, 1853) (Hymenoptera: Vespidae), polyandry in *Apis mellifera*), *Bombus* seem to lack such mechanisms and are susceptible to severe consequences of inbreeding (Cowan and Stahlhut 2004). In *Bombus,* offspring fertilized by diploid male sperm are triploid and are a genetic dead-end. Triploid workers have been reported from wild island populations of *B. muscorum* and *B. jonellus* in the United Kingdom (Darvill, *et al.* 2012). Inbreeding leads not only to diploid male production, but colonies producing diploid males also show slower growth, greater individual mortality and lower worker production (Whitehorn, *et al.* 2009). Although the potential impacts of inbreeding are substantial for haplo-diploids with sl-CSD, diploid males are rarely noted, yet they have been found in both laboratory and wild populations of both stable (Zayed 2009) and severely declining species (Takahashi, *et al.* 2008). Even severely declining populations might lack diploid male production. For example, no diploid males were recovered from a survey of 97 males of the declining species *B. distinguendus* Morawitz, 1869 in the United Kingdom (Charman, *et al.* 2010).

The consequences of inbreeding that are commonly studied in diploid organisms must be analyzed a little differently in haplo-diploid organisms. Often, inbreeding depression is thought to be caused by dominance: the increased presence of deleterious, recessive alleles in inbred populations. In bees, haploid males are an ideal field for selection to act upon and remove such alleles from the population, thus dominance is less likely to be an issue for inbred bees than for inbred, diploid organisms (Zayed 2009). The fitness reduction that is driven by matching sl-CSD alleles can be seen as a special case of overdominance, a reduction in fitness due to homozygosity that is characteristic within some inbred populations. In addition to the observed reductions in fitness ascribable to diploid male production, *Bombus* also exhibit a reduction in

individual and colony fitness that might be due to more classic inbreeding depression. For example, *B. terrestris* queens that mated with their brothers produced new queens that had a lower chance of surviving through the overwintering stage and a lower chance of successfully founding new colonies (Gerloff and Schmid-Hempel 2005).

Yet, there is an inevitable circularity here, with feedback loops that confound discretely relegating genetic characteristics to either cause or effect. Small, genetically homogenous populations are more likely to decline by entering an extinction vortex. Already declining species would be likely to be made up of fragmented populations. Thus, the lower genetic diversity observed might be a result of, rather than a cause of decline. When we detect low genetic diversity or diploid males in populations of bumble bees, we might be detecting declining populations rather than uncovering a cause of decline, yet lower genetic diversity can exacerbate this decline.

Even when entire species are declining, decline is a population-level phenomenon, and some work on island populations has indicated that investigations on bee decline might be more appropriate at the population level, rather than across the entire range of a species. For example, fragmented populations of a declining species in the United Kingdom, *B. sylvarum*, showed lower effective population sizes, heterozygosity and allelic richness than continental populations of the same species, which are considered stable (Ellis, *et al.* 2006). Additionally, island populations of both stable and declining North American species show strong genetic signals typically associated with decline, *i.e.* lower diversity and higher isolation (lower expected heterozygosity, greater differentiation (F_{ST})) , further proof that genetic diversity within species might vary at the population level (Lozier, *et al.* 2011).

All in the family: Declining species are more closely related to one another

Phylogenetic relatedness is another factor that might play a role in the differential decline observed among some species of *Bombus.* Species that are closely related might, by nature of a shared evolutionary history, share traits that unidirectionally influence how they respond to environmental (*e.g.* climate, land-use, pathogen pressure) changes. Phylogenetic clades of plant genera in both the eastern United States and in the United Kingdom have similar abilities to adjust their phenology to track localized climate changes, in spite of geographic and climate change differences between the two regions (Davis, *et al.* 2010). Bumble bee researchers are fortunate that a robust phylogeny has been constructed that includes most (218 of 250 total) recognized species and all 38 subgenera (Cameron, *et al.* 2007).

In North America, the most severely declining bumble bee species are members of the subgenus *Bombus sensu stricto: B. affinis* Cresson, 1863, *B. franklini* (Frison, 1921), *B. terricola* Kirby, 1837 and *B. occidentalis* (Cameron, *et al.* 2011). In Europe, all members of *Subterraneobombus*: *B. distinguendus, B. fragrans* (Pallas, 1771) and *B. subterraneus* (Linnaeus, 1758), are critically declining, and *B. subterraneus* has not been spotted in Britain since 1988 (Williams, *et al.* 2011). A North American member of *Subterraneobombus*, *B. borealis,* Kirby, 1837, has also shown evidence of decline (Colla, *et al.* 2012). Bumble bees that are closely related are likely to share ecological and physiological traits that could influence their susceptibility to environmental factors that drive decline. In bumble bees, this notion is most strongly supported by surveys of their pathogens and parasites. For example, the parasitic tracheal mite *Locustacarus buchneri* (Stammer, 1951) (Trombidiformes: Podapolipidae) seems

to exclusively infest members of the subgenus *Pyrobombus* in North America (*B. bimaculatus, B. impatiens, B. mixtus* Cresson, 1878, *B. perplexus* Cresson, 1863, *B. sitkensis* Nylander, 1848 and *B. vagans* Smith, 1854), suggesting a phylogenetic susceptibility within this group (Goldblatt and Fell 1984; Kissinger, *et al.* 2011). Similarly, members of *Bombus s. s.* in the United States are more likely to harbor infections of the microsporidian pathogen, *Nosema bombi*, than members of the more characteristically stable subgenera *Cullumanobombus* and *Pyrobombus* (Cordes, *et al.* 2012). *Nosema* infections were also common in *Thoracobombus* examined in that study, a subgenus containing two species of concern in North America: *B. pensylvanicus* and *B. fervidus* (Fabricius, 1798) (Colla and Packer 2008). Species decline is not uniform within subgenera, however. For example, the subgenus *Pyrobombus* contains many of the species modeled as exemplarily stable, *B. vosnesenskii, B. bimaculatus, B. impatiens* in North America and *B. jonellus* in the United Kingdom, as well as two species suspected of declining in North America, *B.ternarius* Say, 1837 and *B. sandersoni* Franklin, 1913 (Grixti, *et al.* 2009; Darvill, *et al.* 2010; Cameron, *et al.* 2011; Colla, *et al.* 2012).

Picky eaters go hungry: Declining species are more specialized in their diets

In a survey of 438 native bee species in the Northeastern United States collected over the last 140 years, oligolectic species were more likely to have declined than polylectic species (Bartomeus, *et al.* 2013). Similarly, oligolecty has been associated with declining solitary bee species in Britain since the 1980s (Biesmeijer, *et al.* 2006). Diet specialization might also leave certain species of bumble bees more vulnerable to decline. Although bumble bees are polylectic, they do exhibit species-level differences in their floral preferences. These differences are thought to be mainly driven by the length of their glossae (tongues), which vary widely among species (Medler 1962). These differences in glossa length allow communities of bees to partition

otherwise similar resources, presumably lessening competitive interactions (Inouye 1978). For example, bees with longer glossae have near-exclusive access to nectar from flowers with long corollas and might exhibit a preference for flowers with that morphology. When corolla length and glossa length are well-matched, foraging efficiency increases, and in communities of multiple *Bombus* species, preferences follow glossa lengths (Inouye 1980). Interestingly, long-glossa bumble bees often exhibit a wider diet breadth than their short-glossa congeners (Harder 1985). Bees with long glossae seem to have the advantage in that they can access nectar from both long and short corollas, while those with short glossae are restricted to short-corolla flowers. This asymmetry would suggest that long-glossa species could out-compete shorter-glossa species within a community (Ranta and Lundberg 1980).

The glossa-length driven "preferences" ascribed to bumble bees ultimately depend upon the presence of competition within the bumble bee community under consideration. The plasticity of flower preference has been experimentally shown through removal studies. When bumble bee species are removed from a community, the remaining species become less specialized and will use previously ignored resources (Inouye 1978). Resource partitioning along glossa lengths is thus likely to be more substantial in areas of food limitation, where competitive effects would be more pronounced. In isolated meadow patches in the Rocky Mountains of Colorado, United States, bumble bee communities typically consist of a single representative long, medium and short glossa species, plus a nectar-robbing species that bypasses the limitations of corolla length by chewing a hole in the base of the flower to access nectar (Pyke 1982). Presumably areas of resource excess would be capable of supporting more than one member of each glossa-length class. Indeed, studies of bumble bee communities in Europe

typically show large communities of eight or more species at any given site (*e.g.* Williams 1989; Goulson and Darvill 2004; Fitzpatrick, *et al.* 2007; Goulson, *et al.* 2008b).

Habitat reduction might not be unilateral; some plant species might remain abundant, others might disappear, and new species can become established. This could drive differential stability in bees that depend upon different resource classes. Long-glossa bumble bee species are often considered more at risk of decline than their shorter-glossa congeners. There is some support for this premise in studies of declining bumble bees in Europe. Flowers in the family Fabaceae, such as the clovers (Fabaceae: *Trifolium*), generally have ample nectar and pollen within long corollas. Many legumes have been found to be favored nectar and pollen sources for bumble bees, particularly those with longer glossae (Goulson, *et al.* 2005). In Europe, agricultural practices have shifted away from growing legumes for forage, and the loss of this major floral resource might be contributing to declines in some species of bumble bees. A study of bumble bee communities in Swedish red clover fields found that shorter-glossa species increased in relative abundance compared to longer-glossa species in comparisons over three time periods from 1940 to present day (Bommarco, *et al.* 2012). Similarly, five long-glossa species have declined or been extirpated from red clover fields in Denmark since the 1930s (Dupont, *et al.* 2011). In the United Kingdom, *B. muscorum*, *B. humilis* Illiger, 1806 and *B. ruderatus* (Fabricius, 1775) all have long glossae and show an affinity for Fabaceae pollen; all three are declining (Goulson, *et al.* 2005). The association between glossa length and bumble bee decline has been rejected in other studies, however. When British bumble bee species were present at the same sites and given the same floral choices, nationally declining species were no more specialized in their food choices than their stable counterparts (Connop, *et al.* 2010). Results were similar in a nation-wide survey of declining species of bumble bee in Ireland;

declining species were not characterized by a narrower diet breadth (Fitzpatrick, *et al.* 2007). In a meta-analysis of declining bumble bee species in China, the United Kingdom and Canada, neither glossa length nor diet breadth was associated with declining species (Williams, *et al.* 2009). Species noted as potentially declining in North America include the long-glossa species *B. auricomus* (Robertson, 1903), *B. borealis, B. fervidus, B. fraternus* (Smith, 1854) and *B. pensylvanicus,* but also include the short-glossa species *B. affinis, B. ashtoni* (Cresson, 1864), *B. citrinus* (Smith, 1854), *B. franklini, B. occidentalis, B. terricola* and *B. variabilis* (Cresson, 1872) indicating that factors other than diet specialization might be more important in North American bee decline (Colla and Packer 2008; Grixti, *et al.* 2009; Cameron, *et al.* 2011). Interestingly, many long-glossa species in Europe and in North America are also known to be late-emerging species (Plowright and Laverty 1984; Goulson, *et al.* 2005), an association potentially confounding the search for causes of bee decline.

The rare become rarer: Declining species have smaller range sizes

A general trend of "positive interspecific abundance-range size relationships" has been noted across many taxa (Gaston, *et al.* 1997). Briefly, animals with large geographic range sizes are likely to be more locally abundant than sympatric congeners with smaller range sizes. This also implies the opposite, negative relationship: animals with small ranges are more likely to be locally rare. Rarity itself might predispose species to extirpation. Smaller populations are typically more vulnerable to stochastic events that might drive them to expiration. Similarly, many declining bumble bee species have small range sizes, and this characteristic might help contribute to their decline. The size of a species' historical range throughout Europe is strongly correlated with its recent decline within the United Kingdom alone (Williams 2005). In a comparison of historical and contemporary museum records from eastern North America,

bumble bee species with smaller ranges exhibited greater range contractions and were less persistent than sympatric species with larger range sizes (Colla, *et al.* 2012). This was also the case in Ireland, where rare bumble bee species with the smallest ranges historically were declining the most (Fitzpatrick, *et al.* 2007). In Illinois, many species that were locally extirpated (*B. borealis*, *B. ternarius* and *B. terricola*) or exhibited contemporary declines (*B. affinis* and *B. fraternus*) were historically rare as well (Grixti, *et al.* 2009). *Bombus franklini* historically has one of the smallest ranges of any bumble bee species (Williams, *et al.* 2007), and a petition to protect the species under the Endangered Species Act has recently been filed (Thorp, *et al.* 2010). There are declining species that do not fit this trend, however. Up until recently, *B. occidentalis* and *B. pensylvanicus* had very large ranges throughout North America, yet their ranges have decreased 28% and 23%, respectively, in contemporary times, and these two species are now thought to be declining (Cameron, *et al.* 2011).

It is also possible that the link between range size and rarity is not independent of other hypotheses that have been proposed for the causes of bumble bee decline. One of the possible causes of the relationship between range size and abundance is that species with wider resource breadth, that is bumble bees that are less specialized in their environmental and floral needs, are better equipped to both expand their range and to become locally abundant ("Brown's hypothesis", Gaston, *et al.* 1997). Although bumble bee species with broader diets tend to be locally abundant (Goulson and Darvill 2004), a direct link between diet breadth measured locally and geographic range size has not yet been supported. Small range sizes might also indicate small population sizes, which might suffer from the aforementioned problems associated with lower genetic diversity.

No place to go: Declining species are close to the limits of their climatic niche

Populations of bumble bees that are located at the edge of the range of the species might be more vulnerable to decline. This pattern is hypothesized to stem from the fact that range edges are marginally suitable habitats for organisms and that their persistence there is already tenuous. A British site with lower resource levels at the edge of species' ranges showed only seven of thirteen *Bombus* species and these in lower abundance, than when compared to a nearby site with ample floral resources (Williams 1989). The pressures of subsisting on the periphery of an ideal climatic niche might be alleviated by increased availability of food resources locally, but these populations would remain more vulnerable to decline than their counterparts safely within the climatic niche (Williams, *et al.* 2007). In North America, bumble bees tend to be more abundant in the centers of their ranges, with patchier records at the periphery of the region in which they have been recorded (Plath 1934; Colla, *et al.* 2011). This pattern is also the case in Britain (Williams, *et al.* 2007). Two well-recorded species of declining bumble bees in Europe, *B. distinguendus* and *B. sylvarum*, showed a decline in persistence at the edges of their climatic niches, while a wide-spread and stable species, *B. pascuorum* (Scopoli, 1763), showed no such pattern (Williams, *et al.* 2007). On the other hand, no correlation between range limits and decline was uncovered in a study of Irish bumble bee decline (Fitzpatrick, *et al.* 2007).

The pressures of changing climate might alter the tenuous suitability of habitat on the edge of species' ranges. One species from northeastern Brazil, *B. bellicosus* Smith, 1879, is assumed to be extirpated as its range has contracted southward in recent years (Martins and Melo 2010), consistent with climate change predictions. In the northeastern United States, native bee

species with range limits that were far south showed increased in abundance over the last 140 years, consistent with a prediction of a climate-change driven northward shift in species' ranges in the eastern United States (Bartomeus, *et al.* 2013). Conversely, *B. pensylvanicus* populations were rarer in the northern and more stable in the southern portion of their range in the eastern United Sstates in a recent range-wide survey (Cameron, *et al.* 2011). *B. pensylvanicus* has also become undetectable in the Northern extreme of its range in Ontario, Canada, in spite of its historical abundance in that region (Colla and Packer 2008). In Britain, shifts in rare bumble bee distributions were in opposite directions, with *B. distinguendus* retreating northward and *B. sylvarum* retreating southward (Williams, *et al.* 2007). It is possible that increased variability in weather due to climate change might have non-intuitive effects such as this.

Don't be late: Declining species initiate colonies late in the season

Bumble bee species exhibit phenological variability that might interact with other factors and contribute to their decline. Species that begin their colony cycle late in the season might be at greater risk of decline, and late colony initiation has been associated with species decline in Europe, Canada and China (Williams, *et al.* 2009). In surveys comparing bumble bees in red clover fields in Denmark during the 1930s to surveys conducted in 2008 and 2009, most of the five extirpated species and seven declining species were late emergers with long glossae (Dupont, *et al.* 2011). Similarly in the United Kingdom, the late-emerging species *B. distinguendus, B. humilis*, *B. muscorum*, *B. soroeensis* (Fabricius, 1776) and *B. sylvarum* are all notably declining (Goulson, *et al.* 2005). This seems to hold true in the eastern United States as well, where some (*B. auricomus*, *B. fervidus* and *B. pensylvanicus*), but not all, of the bumble bee species in decline were reported to begin their colony cycles later in the season than most of

their congeners (Colla, *et al.* 2012). Few details are known about the phenology of most bumble bee species, and species cycles are likely to vary geographically.

It is important to note that classification of emergence times as "Early" and "Late" might lead to some confusion, as these classifications are relative among local taxa and might vary considerably by location. Although few taxa have been studied in depth, it seems that the available evidence points to ambient temperature as the cue used by queens to break diapause, but species differ in their temperature thresholds (Alford 1969). This variability leads to differential colony initiation times among bumble bee species in what Frison (1926) termed "appearance-succession". In as study of 14 species in Alberta, Canada, only three weeks separate the earliest nest-founding species, *B. frigidus* (Smith, 1854), from the latest nest-founding species, *B. appositus* (Cresson, 1878) (Richards 1978). Kearns and Thomson (2001) list *B. pensylvanicus* as an early emerging species, yet *B. pensylvanicus* is one of the last species to emerge in Boston, with nests initiated in June (Plath 1923), rather than in April or May as in the cases of *B. bimaculatus* and *B. impatiens* there (Plath 1922). In Boston, *B. bimaculatus* and *B. impatiens* initiate nests two weeks before *B. auricomus* (Frison 1926). Similarly in Ontario, *B. auricomus* queens emerge in early May (Colla and Dumesh 2010) and in Alberta they emerge in late May–Early June (Hobbs 1965). While this has been deemed "late" by some (Colla, *et al.* 2012), *B. auricomus* also emerges in April–May in Northwest Arkansas (see Chapters II and V, herein), where, along with *B. bimaculatus*, it is among the earliest species to initiate colonies in the spring. The span of bumble bee appearance-succession is 10 weeks in Northwest Arkansas (see Chapter V), as compared to three weeks in Alberta, Canada (Richards 1978), six in Southern England (Goodwin 1995) or seven in Southern Ontario (Colla and Dumesh 2010).

If colony founding is largely prompted by temperature, local adaptation seems likely. In England, *B. jonellus* is an early species that completes its cycle by July, while north in the Hebrides off of Scotland, it is one of the last species to initiate colonies, which then persist until fall (Goulson, *et al.* 2006). In Alberta, the variety of *B. fervidus* formerly known as *B. californicus* Smith, 1854 (Williams 1998), emerges at a calendar date later than *B. fervidus*, but conditions associated with spring (*e.g.* ambient temperature, flowering) occur two weeks later in the foothills where the *californicus* variety is found than they do in the flat, prairie land in which *B. fervidus* proper occurs (Hobbs 1966). Yet another complication in classifying appearance-succession among bumble bee species is that queen emergence is not typically a discrete phenomenon for a particular species. Large numbers of queens of some species (*e.g. B. lapidarius* (Linnaeus, 1758), *B. lucorum* (Linnaeus, 1761) and *B. terrestris*) have been continuously observed seeking nests over periods as long as six weeks (Kells and Goulson 2003). Unless emergence time is measured over the entire range of a species (and at multiple points in history), or a less subjective measure of emergence time is used (*e.g.* degree day models), bumble bee conservation workers would be wise to avoid making broad generalizations of species characteristics that are based only upon local phenological data.

There are many plausible explanations for why late queen emergence might be related to a greater propensity to colony failure and species decline. Heat and drought conditions common during summer might indirectly impact colonies established later in the season through loss of forage plants, factors that might be exacerbated under climate changes (Rasmont and Iserbyt 2012). Parasites and pathogens tend to have seasonal cycles, and bumble bee queens that forage later in the season might be more likely to be parasitized by conopid flies, as was the case for *B. fervidus* queens in Alberta (Hobbs 1966) and other species in Maine (Heinrich 2004). One factor

that is likely overlooked in studies of niche differences in bumble bees is that of nesting site availability. Bumble bees nest in existing cavities, typically abandoned vertebrate dens with constricted entrances, both above and below ground. Nests are typically difficult to locate, and there is much to be discovered about nesting and overwintering biology of bumble bees. In one of the few studies with a substantial number of observations of queen nest-founding (11 species, 147 queens), seven species exhibited strong differences in the types of habitats that queens selected (Svensson, *et al.* 2000). Nest niches might also be important factors in the partitioning of bumble bee communities, and nest sites might be limiting at least in some areas. Usurpation of nests is common (9–11%), and species seem to overlap in their preferences for nest characteristics (*e.g.* landscape of location, above or below ground) along phenological lines (Richards 1978). Species that establish nests later in the season might find fewer options available. On the other hand, early nesting species are often presented with the volatility of spring weather, with cold, wet conditions leading to the failure of many colonies that are begun early in the season (Hobbs 1967; Harder 1986). The appearance-succession of bumble bee species presents an interesting puzzle that suggests the presence of potential trade-offs between nesting early and nesting late that differentially affect success among species. For late-starting species, the length of the colony cycle might also influence the likelihood of reproductive success.

Don’t take too long: Declining species have longer colony cycles

For native bees as a whole, shorter adult active times are correlated with species decline in the northeastern United States (Bartomeus, *et al.* 2013). As they are social insects, the situation in bumble bees is quite different, however. The development of a bumble bee colony proceeds from nest initiation by a single queen to the production of next year’s reproductives. In

the interval, broods of workers must be produced in numbers sufficient to provide the developing larvae with enough food to become queens. Feeding marks the main difference between worker and queen development, with frequent and more abundant larval feeding giving rise to new queens (Plowright and Jay 1977). Sociality sets bumble bees apart from most native bees and dictates that colonies have a longer adult activity period than most solitary individuals, which in turn requires that floral food resources are available over a longer season. While bumble bees have relatively long periods of adult activity when compared to most solitary species, there is also a great deal of variation among species of *Bombus*.

The timing of colony initiation might also dictate colony duration and ultimate colony size, as early emerging species might have the opportunity to produce more worker broods over a growing season prior to completing the colony cycle with reproductive production (Hobbs 1967). Thus, the combination of late emergence and long colony cycles might leave some species particularly vulnerable to shifting environmental conditions (Williams, *et al.* 2009). This hypothesis seems contradicted by the life history and abundance of *B. pascuorum* in the United Kingdom. It is not only a flagship stable species, but also has one of the longest cycles and latest colony initiation times of any species studied in depth there (Goodwin 1995).

A link between specialization and colony cycle length has also been suggested, with species with short colony cycles having a greater tendency for diet specialization than those with long colony cycles (Goulson and Darvill 2004). This led to the suggestion that long-cycle generalists might be less vulnerable than short-cycle specialists, but when examined explicitly, this has not been shown to be the case (Williams, *et al.* 2009). It seems likely that species with short colony cycles have a lower diversity of plants available to them at any given site than those

with longer cycles, as individual plant species tend to have bloom cycles that are generally shorter than bee colony cycles.

Too close to home: Declining species have smaller foraging ranges and dispersal distances

Species differ in their foraging ranges, queen dispersal distances (*e.g.* Darvill, *et al.* 2004; Osborne, *et al.* 2008; Wolf and Moritz 2008; Knight, *et al.* 2009; Carvell, *et al.* 2011) and in their average and maximum homing distances (Walther-Hellwig and Frankl 2000). An abundant British species, *B. pascuorum* responded to its landscape only at a 250 m scale, suggesting that its foraging range is quite small compared to its congeners *B. terrestris* and *B. lapidarius* (3,000 and 2,750 m, respectively) (Westphal, *et al.* 2006). A species with a propensity for long foraging distances might be more capable of finding food sources in times of local scarcity. Differences in dispersal distances of reproductives might also influence a species' resilience, as species with greater dispersal distances might be better at maintaining gene flow across fragmented habitats. There is at least one example of a correlation between dispersal distance and species' decline in Britain (Darvill, *et al.* 2010). The stable *B. jonellus* is a habitat specialist that has likely adapted to long-distance dispersal in response to the patchy distribution of the heathland habitats with which it is associated. In contrast, *B. muscorum*, a declining species, evolved in coastal habitats that are continuous. Populations of *B. muscorum* were shown to be isolated from other populations only 3.2 km away, yet no populations of *B. jonellus* were isolated at distances less than 7.1 km. The increased gene flow among *B. jonellus* populations might help explain how it is maintaining stability while its congener, *B. muscuorum*, is declining.

Stranger danger: Exotic bees are driving declines through competition, hybridization and the introduction of novel pathogens

Bombus terrestris has been introduced to many areas outside of its range as an agricultural pollinator and the impacts of the naturalization of this species in novel areas are diverse and frightening. In Japan, *B. terrestris* competes with native *Bombus* species for both floral and nesting resources and tends to produce more reproductives than local species (Matsumura, *et al.* 2004; Takahashi, *et al.* 2008). Worse yet, *B. terrestris* interbreeds with the native species *B. hypocrita* Pérez, 1905 in Japan, and the resulting hybrid females are inviable (Kanbe, *et al.* 2008). Nearly 30% of 281 field-caught queens examined had *B. terrestris* sperm in their spermathecae, indicating that hybridization between the introduced *B. terrestris* and native *B. hypocrita* has the potential to severely impact populations of the native Japanese species. Exotic strains of the tracheal mite, *Locustacarus buchneri* (Stammer, 1951) (Trombidiformes: Podapolipidae) have been also introduced to Japan with the importation of commercial *B. terrestris* (Goka, *et al.* 2001). These exotic mite haplotypes have since been recovered from native Japanese bees, although the impact of the exotic strains on native species remains unknown (Goka, *et al.* 2006).

Even when native species are used, commercial *Bombus* trafficking might have severe impacts on native populations of bumble bees. The "pathogen-spillover hypothesis" suggests that traffic in commercial bumble bees is increasing the prevalence of disease among native *Bombus* in areas by introducing novel strains and species of pathogens into native populations (Colla and Packer 2008). The prevalence of *Nosema bombi* Fantham and Porter, 1914 (Microsporidia: Nosematidae), has been associated with species decline in North America (Cameron, *et al.* 2011). It has been hypothesized that bumble bee declines in North America are the result of the

introduction of a novel, European strain of *N. bombi* via commercial rearing facilities, but thus far there is no empirical support for this scenario (Williams and Osborne 2009; Sokolova, *et al.* 2010; Kissinger, *et al.* 2011; Koch and Strange 2012). In Ontario, *Crithidia bombi* Gorbunov, 1987 (Kinetoplastida: Trypanosomatidae) pathogen loads and infection prevalence were higher for wild foragers near greenhouses and decreased with increasing distance from sites with known commercial *Bombus* exploitation (Otterstatter and Thomson 2008). Modeling based on the density of greenhouses in North America suggests that *B. terricola* and *B. pensylvanicus* populations are less likely to persist in areas with high commercial *B. impatiens* use (Szabo, *et al.* 2012). Thus far, pathogens have not been linked to bumble bee declines in Britain (Williams, *et al.* 2007). However, a survey of commercial *B. terrestris* colonies from three European producers revealed that 77% of these colonies (*i.e.* bumble bee specimens or the provided pollen supplies) were infested with pathogens, including known *Bombus* pathogens, in spite of a legal requirement that these colonies be certified "pathogen-free" prior to shipment (Graystock, *et al.* 2013).The pathogens found in this study included *Apicystis bombi* (Liu, Macfarlane and Pengelly, 1974) (Apicomplexa: Neogregarinorida), *C. bombi* and *N. bombi*, as well as several honey bee pathogens, *N. ceranae* Fries, 1996 *N. apis* (Zander, 1909), deformed wing virus, European foulbrood and American foulbrood. Furthermore, many of these were pathogenic to laboratory bumble bees, including *N. ceranae,* a microsporidian typically associated with honey bees.

Because they often share the same floral resources (Thomson 2006), native bumble bees are exposed to many pests and pathogens that are associated with the ubiquitous, non-native honey bee, *A. mellifera.* Some of these are pathogenic to native bumble bee species and have been discovered among wild populations, although the potential impacts of many honey bee

pathogens on bumble bees are largely unknown. Individuals exhibiting deformities and testing positive for deformed wing virus, a honey bee pathogen, have been found in commercial colonies of *B. terrestris* and wild colonies of *B. pascuorum* in Germany (Genersch, *et al.* 2006). Surveys of RNA viruses in Pennsylvania and New York in the United States have uncovered a number of honey bee viruses from wild *Bombus* foragers, including Israeli acute paralysis virus, deformed wing virus, sacbrood virus, Kashmir bee virus and black queen cell virus (Singh, *et al.* 2010). Whether or not these viruses are pathogenic to bumble bees remains unknown except in the aforementioned case of deformed wing virus (Meeus, *et al.* 2011). In Argentina, molecular screening revealed the presence of *N. ceranae* in three native bumble bee species, *Bombus atratus* Franklin, 1913 *Bombus morio* (Swederus, 1787) and *Bombus bellicosus* (Plischuk, *et al.* 2009). The invasive small hive beetle *Aethina tumida* Murray, 1867 (Coleoptera: Nitidulidae) is an imported pest of honey bees that has been recorded invading commercial colonies of *B. impatiens* since its arrival in the United States (Spiewok and Neumann 2006). Laboratory studies show that the small hive beetle can locate and utilize *B. impatiens* nests readily, but that the bumble bees exhibit defensive and hygienic behaviors that lessen the impact of infestation on the colony (Hoffmann, *et al.* 2008). The impact of small hive beetle infestations on naturally-occurring bumble bee nests is currently unknown. In addition to carrying pathogens, honey bees might compete with bumble bees for nectar and pollen when these resources are scarce. When *A. mellifera* hives and *B. occidentalis* colonies were placed in a California nature reserve, *B. occidentalis* colonies fared increasingly better (*i.e.* increased pollen foraging and higher production of larvae and end-of-season reproductives) with increasing distance from *A. mellifera* hives (Thomson 2004). The manifold impacts of introducing non-native bee species into native bumble bee communities remain largely unexplored.

Summary

As this review suggests, there are many potential drivers of bumble bee decline, and no single cause has been identified to date. Species-level differences are likely to result in differential responses to external pressures, and broad generalizations might obscure the true causes of decline. Additionally, many of the factors impacting bumble bee populations might also play a role in the declines of other native bees. In the work that follows, I address a number of these potential causes of bumble bee decline by examining Arkansas *Bombus* species in detail. There are six *Bombus* species that are common in the state, including three species that have been the subject of recent investigations of *Bombus* decline: *B. bimaculatus*, *B. impatiens* and *B. pensylvanicus*. In Chapter II of this work, I investigate changes in the occurrence of Arkansas *Bombus* species over the last 50 years in order to characterize local species as stable or declining. In Chapter III, I characterize the genetic diversity of six species in Arkansas and Tennessee, and in Chapter IV, I report the occurrence of diploid males in the region. Chapter V includes detailed information about the ecological differences among *Bombus* in Northwest Arkansas that addresses many of the proposed causes of decline in this review. Additionally, Chapter V places *Bombus* within a community context, comparing abundance, phenology and host plant use among *Bombus*, *Xylocopa virginica* and *Apis mellifera.*

C. Contrasts of Diversity and Distribution in Carpenter Bees

Carpenter bees in the genus *Xylocopa*, like bumble bees, are large-bodied, generalist bees. Although carpenter bees share floral communities and seasonality with bumble bees where they co-occur, there are sharp differences between the two groups. Bumble bees are classified as primitively eusocial, *i.e.* they have obligatory colonies with overlapping generations, cooperative brood care and division of labor, but queens have a solitary phase and there is no morphological

distinction among castes. Carpenter bees, on the other hand, at most show a tendency toward parasocial behavior, with two females of overlapping generations often sharing a nest and cooperatively caring for brood by performing different tasks (Michener 1990). Unlike bumble bees which enjoy a reputation as an important group of pollinators worthy of conservation in the United States *(e.g.* Cameron, *et al.* 2011), carpenter bees are generally considered structural pests (*e.g*. Barrows 1980) in spite of their proven pollination services (*e.g*. Liu and Koptur 2003; Sadeh, *et al.* 2007). They show different climatic affinities as well. Carpenter bees are more speciose in tropical and Neotropical areas (Hurd and Moure 1963), whereas bumble bees are more common in temperate and montane regions (Williams, *et al.* 2014). The contrast between the two groups that is perhaps more relevant here, however, is that rather than showing evidence of species decline worldwide, the *Xylocopa* appear to be expanding their ranges (but there are exceptions *e.g.* members of the subgenus *Lestis* (Steen and Schwarz 2000).

As early as the 1950s, *X. violacea* (Linnaeus, 1758) was noted as expanding its range throughout continental Europe (reviewed in Hurd and Moure 1963), and it is thought to have established itself in Britain since 2007 (Bees Wasps and Ants Recording Society 2010). Although there is no true evidence thus far, researchers in Canada anecdotally report that *X. virginica* is becoming more common and widespread at the northern extent of its range (reported in Skandalis, *et al.* 2011), consistent with poleward range expansions predicted by climate change (Hickling, *et al.* 2006). Another apparent expansion has been noted in *X. micans* Lepeletier, 1841, which has recently been found in areas far north of its expected range in the southeastern United States (Warriner 2010; Tripodi and Szalanski 2011). These examples seem to fit a pattern of range expansion, in which new populations extend the boundaries of a species' range. The ecological factors driving these changes are unknown at present, but it is tempting to

ascribe these distribution shifts to contemporary climate changes. However, the *Xylocopa* are particularly prone to anthropogenic introductions to areas outside of their natural ranges through the human-mediated transportation of wood. *Xylocopa virginica* has been found in Colorado, in the western United States far out of its native range, on two occasions, although it is not thought to have established there (Scott, *et al.* 2011). The availability of suitable nesting materials in human developments might have allowed *X. tabaniformis orpifex* Smith, 1874 to extend its range into lower elevations in California (Hurd 1955). Thus, anthropogenic factors might also play a role in *Xylocopa* distribution shifts. However, animal distributions are governed by more than contemporary ecological processes, and past environmental conditions and ecological processes have played an important role in shaping species distributions, although this historical element is often ignored (Brown, et al., 1996). The evolutionary history of bees in eastern North America might offer insights into their distributions that are not obvious on the contemporary ecological level.

The two species of large carpenter bees in eastern North America, *X. virginica* and *X. micans*, offer a unique opportunity for comparative phylogeography that might provide insight into the long-term history of bee distributions in the region. Although they co-occur in the southern extent of their ranges, the range-size disparity between *X. micans* and *X. virginica* is striking. Worldwide, the distributions of carpenter bees are thought to be governed by climatic factors such as temperature and precipitation (Porter 1981; Watmough 1983). Although the *Xylocopa* are more common in tropical climates, some species in temperate areas have extensive ranges, consistent with Rapoport's Rule (Brown, *et al.* 1996). Of the 29 species within the subgenus *Schonnherria*, only *X. loripes* Smith, 1874 (Arizona and Mexico) and *X. micans* occur outside of the Neotropical region (Hurd 1955, 1978). Until recent reports of *X. micans* in

Arkansas (Warriner 2010; Tripodi and Szalanski 2011), the distribution of this species was restricted to the warmer regions of the southeastern United States along the Atlantic coast south to Guatemala (Hurd 1955). Although *X. micans* appears to enter a quiescent period in colder months, diapause has not been conclusively shown in this species (Porter 1981). Many tropical insects are incapable of diapause and are not expected to be found in regions that fall below -4ºC, because tissues fatally freeze at this temperature (Parmesan, *et al.* 2005). This suggests that the northern range limit of *X. micans* could be governed by temperature tolerances. Conversely, *X. virginica* is widely distributed east of the 100th meridian, including regions north of the -4°C isotherm (Hurd 1955; Skandalis, *et al.* 2011) All five members of the subgenus *Xylocopoides* are Nearctic in distribution, but only *X. virginica* appears in the East. *Xylocopa virginica* adults overwinter in diapause, emerging in spring as temperatures reach ~23°C (Balduf 1962). However, they are capable of some endothermy and can raise their body temperatures 13.5–16.4°C above ambient air temperatures, which allows them to forage at temperatures as low as 15°C (Baird 1986). Preliminary climate models of their contemporary distribution show that the northern range of *X. virginica* is limited by temperature, and the western range is limited by precipitation (Skandalis *et al.* 2011).

Contemporary distributions also reflect past colonization events. It is hypothesized that *Xylocopoides* and *Schonnherria* diverged prior to independently colonizing North America by crossing Beringia over 34 million years ago (Leys, *et al.* 2002). By examining their morphology and preferred nesting sites, Hurd (1956) proposed that the *Schonnherria* were already established in the New World when *Xylocopoides* arrived. Both species of *Xylocopa* in eastern North America were later subjected to the same climate changes during the Pleistocene glaciation cycles, which reached their maximum about 12,000 years ago (Soltis, *et al.* 2006). During this

period, both groups were forced south by cooler climates and are hypothesized to have independently recolonized North America from Central America as the glaciers receded and temperatures rose (Leys, *et al.* 2002). It is during this period that the contemporary ranges of each species were determined. *Xylocopa virginica* occupied most of the eastern half of North America, while *X. micans* only established in the southeastern extreme. This pattern could simply reflect differential climatic tolerances between the two species, but other explanations are also plausible.

Post-glacial colonization patterns might have been influenced by competitive interactions among species that have shared ecological niche requirements. Hewitt (2000) proposed that, among organisms with similar niches, initial colonizers residing in refugia closer to the ice boundary could block secondary colonizers from expanding their ranges by occupying available niches more quickly. Initial colonists would expand rapidly and occupy large geographic ranges. This rapid expansion would reduce overall genetic diversity in such taxa. In contrast, secondary colonists with similar resource needs that survived the glaciations in more distant refugia would find it difficult to occupy these already inhabited areas. These taxa should exhibit smaller geographic ranges but have higher genetic diversity and potentially greater population structure (Hewitt 2000; Douglas, *et al.* 2009). The contemporary distributions of *Xylocopa* in eastern North America show a pattern that suggests that *X. virginica* could have been an initial colonizer such as this, followed secondarily by *X. micans*.

Applying Hewitt's (2000) hypothesis to explain range disparity in the *Xylocopa* of eastern North America offers a simple, testable prediction. As an initial colonizer, *X. virginica* should exhibit lower genetic diversity than *X. micans.* However, *X. virginica* is polytypic, with three described subspecies, unlike the monomorphic *X. micans* (Hurd and Moure 1963). Insects

with large geographic ranges are often polymorphic across their ranges, and this can often be attributed to both environmental plasticity and evolutionary history (*e.g.* Vane-Wright and Tennent 2011; Kodandaramaiah, *et al.* 2012). The morphological variation in *X. virginica* might indicate higher diversity and population structuring, contrary to expectations under Hewitt's (2000) hypothesis. Then again, perhaps the subspecies of *X. virginica* represent lineages derived from multiple glacial refugia, some of which could have blocked the post-glacial advance of *X. micans*.

A trinomial designation should allow certain predictions to be made and tested that are distinct from those that would be suggested by a singular name for a group (Barrowclough 1982). In the case of *X. virginica*, the three subspecies suggest three lineages, with *X. v. texana* Cresson, 1872 originating in Texas, *X. v. krombeini* Hurd, 1961 stemming from southern Florida and the nominal *X. v. virginica* occurring in between. Under the best of applications, the trinomen acts as a signpost for further investigations (Mayr 1982), however, incorrect classifications can just as readily lead to false inferences and misdirect both scientific and conservation efforts (Zink 2004). The subspecies of *X. virginica* have not been addressed since the description of the most recently described subspecies, *X. v. krombeini* (Hurd 1961). The subspecies designated within *X. virginica* should reflect distinct lineages in order to be informative, thus a critical examination of the morphology of these taxa was undertaken in this work. In Chapter VI, I revisit the morphology of the three named subspecies of *X. virginica* in order to clarify their taxonomic status. In Chapter VII, I apply phylogeographic analyses to explore the three-point radiation hypothesis suggested by the distributions of the three *X. virginica* subspecies. I also compare the genetic composition of *X. virginica* and *X. micans* throughout their ranges to explore post-glacial colonization hypotheses. The *Xylocopa* of eastern

North America offer a unique opportunity to explore historical factors that might have governed bee distributions in the region.

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II. THE BUMBLE BEES OF ARKANSAS, FIFTY YEARS LATER

A. ABSTRACT

Many species of bumble bees (Hymenoptera: Apidae: *Bombus* Latreille, 1802) are declining throughout their ranges, yet detecting declines can be difficult when historical survey data are lacking. In this work, contemporary data is compared to a 1965 survey to detect changes in bumble bee distributions throughout Arkansas nearly fifty years later. Using county-level records as a point of comparison to look for changes in state-wide occurrence among species over time, I find that state-level changes reflect national trends. Contemporary *Bombus bimaculatus* and *B. impatiens* records have more than tripled, while *B. pensylvanicus* records show a decline to 60% of historical levels. Although *B. fervidus* has been infrequently reported in the state, misidentifications might have led to an overestimation of the state's species richness. In addition to an updated assessment of the bumble bees of Arkansas, I also provide new, localized information on the seasonal phenology and plant preferences of each species that can be used to guide conservation efforts.

B. INTRODUCTION

Many species of bumble bees (Hymenoptera: Apidae: *Bombus* Latreille, 1802) are thought to be declining throughout their ranges in both North America (Cameron, *et al.* 2011; Colla, *et al.* 2012) and throughout Europe (Fitzpatrick, *et al.* 2007; Williams, *et al.* 2009; Dupont, *et al.* 2011). Contemporary resampling techniques have provided evidence for bumble bee declines in Illinois (Grixti, *et al.* 2009; Lozier and Cameron 2009), Ontario, Canada (Colla and Packer 2008), Denmark (Dupont, *et al.* 2011) and Sweden (Bommarco, *et al.* 2012). Few locations are fortunate enough to have detailed historical surveys of bumble bees, however, and other methods must be employed to use historical data to determine the present status of

vulnerable species. Detecting species declines can be difficult, especially in regions that lack historical survey records with which to compare contemporary data.

The use of specimen records in museum holdings offers an alternative method of detecting change over time (Shaffer, *et al.* 1998). Typically, these studies use records of specimens collected throughout the entire range of a species and compare the geographic occurrence or relative abundances across time periods to identify population changes. However, declines might be heterogeneous across a species' range, and smaller-grain assessments could yield conservation recommendations that are easier to implement (Hunter and Hutchinson 1994). Conservation planning in the United States often occurs at a local (state, county city) level delimited by political boundaries (Huber, *et al.* 2010).

There are no known surveys of bumble bee abundance in Arkansas with which contemporary surveys can be compared. However, in 1965, Chandler and McCoy produced a survey of the bumble bees of Arkansas based on state-wide collecting efforts and the museum holdings at that time (Chandler and McCoy 1965). The authors reported the counties in which each species was recorded but gave no quantitative indication of abundance. Here, I use county records as a point of comparison to look for changes in state-wide occurrence among bumble bee species over time. It is not uncommon for historical records to contain only county-level locality data, and a county-level-comparison approach has been used to detect declines in other organisms such as amphibians in California (Fisher and Shaffer 1996). The declining status of many British bumble bees was first detected using vice-county-level records (Williams 1982). Szabo and colleagues (2012) also used a similar census-unit approach to determine the persistence of three *Bombus* species throughout their ranges in North America.

In this work, I compare historical and contemporary Arkansas county records to determine the changes in state-wide occurrence of bumble bees. Additionally, I provide updated taxonomic information and ecological details for each species recorded in Arkansas, including new, localized information on the seasonal phenology and plant preferences of each species that can be used to guide conservation efforts.

C. MATERIALS AND METHODS

The 75 Arkansas counties range in size from 1411–2731 km^2, with an average area of 1836 ± 335 km^2 SD. Each bumble bee species was recorded as present or absent from each county in two periods: historical and contemporary. The historical period included all records through 1965, the publication date of the last Arkansas bumble bee survey (Chandler and McCoy 1965). The contemporary period included all records in the period 2000–2013. This range was chosen to occur after the initial detection periods of *Bombus* decline throughout North America (*e.g*. 1988: *B. franklini* (Frison, 1921); late 1990s: *B. occidentalis* Greene, 1858; 1998: *B. affinis* Cresson, 1863,Committee on Status of Pollinators in North America 2007). New state distribution data for both periods were obtained from holdings at the University of Arkansas Arthropod Museum (UAAM), specimens from a 2011–2013 citizen science survey and our own collection efforts during 2010–2013. Specimens were identified to species using the keys and descriptions of Mitchell (1962) and Chandler and McCoy (1965), and vouchers are deposited in UAAM. Sampling effort within each of the time periods was compared by generating species accumulation curves for each period in the R package *vegan* v.2.0-9 using 1,000 permutations (Oksanen, *et al.* 2013; R Core Team 2014). Changes in the state-wide occurrence of each species were qualitatively assessed with comparisons of the proportion of sampled counties in which a species was observed for each period.

Natural history information for each species was determined from field surveys conducted every other week at 13 sites in Washington, Benton, Carroll, Boone and Madison Counties in Northwest Arkansas between March and October in 2010–2013 as part of a broader survey (see Chapter V). Surveys were conducted by a single observer in non-linear transect walks (Silveira and Godínez 1996; Connop, *et al.* 2010) over 30-min. increments during fair weather (12–39°C). All foraging *Bombus* specimens were collected using an aerial net, and specimens were either identified in the field or retained as vouchers. Activity periods were determined from these surveys using adults of all castes combined. Both the extreme occurrences ("earliest" and "latest") and the dates encompassing 80% of observations ("majority") are reported. Species in which the majority active period begins before mid-summer (mid-June) are considered early-emerging species; those that begin after mid-summer are considered late-emerging species. Activity periods were then classified as short (<63 days), intermediate (63–77 days) or long (>77 days) based on equal intervals across the majority span of observations. Because of their ecological importance in food choice, the worker-glossa lengths of each species are also included. Following the recommendations of Harder (1982), glossal length (length of the glossa between the basal sclerite and the terminus of the flabellum) was deemed more representative of the functional tongue length of *Bombus*, and glossal measurements reported by Medler (1962) are reported as glossa lengths here. The average worker glossa length for each species was then categorized as short (<5.0 mm), medium (5.1–6.0 mm), or long (>6.0 mm). The plant species or genera encompassing at least 75% of nectar and pollen foraging observations of each *Bombus* species over the survey period were noted as preferred plants, and these are listed in order of declining number of observations. Plant identifications to species were conducted in the field and with photographic vouchers using an Arkansas-specific key (Smith 1994), known

distributions (Kartesz and The Biota of North America Program 2013) and a regional photographic field guide (Kurz 2010). In some cases, identification to plant species was not possible, and these records were left at the level of genus (n=110, 9.6%).

D. RESULTS

The previous Arkansas survey yielded 68 records of seven species in 35 counties (Chandler and McCoy 1965). All but nine of these had representatives from the historical time period present in the UAAM collection, and an additional 13 county records from the historical period were obtained from the UAAM collection (years ranging from 1885–1965, n=217). Seven species were recorded in 39 Arkansas counties throughout the historical period: *Bombus auricomus* (Robertson, 1903), *B. bimaculatus* Cresson, 1863, *B. fraternus* (Smith, 1854), *B. griseocollis* (DeGeer, 1773), *B. impatiens* Cresson, 1863, *B. pensylvanicus* (DeGeer, 1773) and *B. variabilis* (Cresson, 1872)**.** For the contemporary period (2000–2013), 92 county records of six species in 36 counties were available. Of these, 28 were confirmations of historical records, and 75 were new county records. All species observed in the historical period were observed in the contemporary period with the exception of *B. variabilis.* Only seven records captured information in the years between our historical and contemporary periods (1966–1999), and each is listed in the species accounts that follow. County-level occurrences of each species within the historical and contemporary periods are shown in Figure II.D.1.A–G. Twenty-two of the 75 counties in Arkansas had no records from either period (Fig. II.D.1.H). Two anomalous records of western species were among the specimens deposited in UAAM: *Bombus occidentalis* (Green, 1858) and *B. vosnesenskii* Radoszkowski, 1862 both collected in the 1980s in Washington county by the same collector. Because this collector had also deposited specimens from the western United States, where these species are found, I assumed that these were mislabeled,

rather than truly collected so far out of their natural range. Although the species *B. fervidus* (Fabricius, 1798) has been reported as occurring in the state (Franklin 1912; Chandler and McCoy 1965; Warriner 2011), I found no evidence of its presence in Arkansas. This is discussed further in the *B. fervidus* section below.

Sampling effort differed between the historical and contemporary periods as evidenced by rarefied species accumulation curves (Fig. II.D.2). These curves show the number of species recorded as a function of the number of sampled counties and are constructed by randomly resampling the data (n=1000 samples). Adequate sampling is expected to result in a flattened curve, while curves with a steep gain reflect data that are under sampled. Sampling did not reach an asymptote in the historical period, suggesting that the state might have been under sampled during this period. During the contemporary period, species richness showed an asymptote early within the number of sampled counties, indicating that the sampling effort was sufficient to capture state-wide species richness.

Most species showed an increase in county occurrence between the historical period and the contemporary period (Fig. II.D.3). *Bombus bimaculatus* and *B. impatiens* records increased three-fold; *B. auricomus* nearly doubled and *B. griseocollis* showed an increase of about one-third. *Bombus fraternus* remained virtually unchanged. *Bombus pensylvanicus* records decreased by 61%. *Bombus variabilis* was not recorded in any counties in the contemporary period.

E. DISCUSSION

Establishing whether or not species are declining or stable is a challenge for species such as *B. fraternus* and *B. variabilis* that are rare throughout their ranges. The relative rarity of *B. fraternus* provides a good example of how site-specific confirmations of the presence of an

uncommon species might lead to erroneous conclusions about the local conservation status of a species. In this study, only four of the 14 historical records of *B. fraternus* were confirmed with contemporary records, yet its state-wide occurrence remained unchanged. An analysis of contemporary persistence based on confirmations at particular localities would indicate a steep decline (>70%), yet our analysis of county-level occurrence suggests that there has been little change in the species within the state. Although *B. fraternus* is widely distributed throughout the Southeastern and Midwestern United States, its relative rarity seems consistent throughout its range (Williams, *et al.* 2014). Over all time periods, the relative abundance of *B. fraternus* remained below 1% in a survey of museum records of all *Bombus* occurring in the eastern United States (Colla, *et al.* 2012). Similarly, *B. fraternus* accounted for less than 2% of all *Bombus* records in Illinois, regardless of the sampling period (Grixti, *et al.* 2009). Rare species are often the center of conservation attention, but detecting declines in such species will require novel approaches to overcome innate statistical difficulties (Strayer 1999).

The number of county records of *B. bimaculatus* and *B. impatiens* has more than tripled between the historic and contemporary periods, while the number of county records of *B. pensylvanicus* has declined to 61% of historical levels (Fig. II.D.3). These changes are consistent with surveys across eastern North America that have examined these three species using relative abundance methods. Rather than comparing count data, relative abundance methods compare the percentage of samples that belong to each category of interest. For example, Cameron, *et al.* (2011) found that among sampled species, the percent of records of both *B. bimaculatus* and *B. impatiens* nearly doubled between historical (1900–1999) museum records and contemporary (2007–2009) sample periods. In a comparison of 14 species in Ontario, the relative abundances of *B. bimaculatus* and *B. impatiens* more than doubled between surveys in the early 1970s and

those in the mid-2000s; *B. pensylvanicus* was not present at all in the later survey (Colla and Packer 2008). Similarly, in a study of 21 Eastern North American species that compared historical (1864–1990) and contemporary (1991–2009) museum records, *B. bimaculatus* and *B. impatiens* were persistent at sites throughout their ranges and exhibited an increase in relative abundance, while *B. pensylvanicus* was absent from 66% of its former range, although it showed no change in relative abundance (Colla, *et al.* 2012). Our county-level occurrence data show that in Arkansas, *B. bimaculatus*, *B. impatiens* and *B. pensylvanicus* exhibit the same temporal trends that have been observed throughout their ranges.

Species-level differences in ecological characteristics and requirements might help explain why some species are faring well, while others are declining. Late emergence times and long glossae have been cited as characteristics shared among some declining species, particularly in Europe (Bommarco, *et al.* 2010; Dupont, *et al.* 2011), but also in North America (Colla, *et al.* 2012). Bees with late emergence times have less time to grow their colonies to the size necessary to produce new reproductives before the end of the season. This can leave species with long activity periods particularly vulnerable to colony failure before next season's reproductives are produced (Williams, *et al.* 2009). Of the three species with late emergence times in Arkansas, *B. impatiens* has increased, *B. pensylvanicus* has decreased and *B. fraternus* has not changed between the historical and contemporary periods (Fig. II.D.3). Both *B. pensylvanicus* and *B. fraternus* have long active periods as well (82 and 92 days, respectively). Species that require a lengthy period of stable floral resources to successfully rear reproductives might be more vulnerable to colony failure during seasonal fluctuations in habitat quality (Williams, *et al.* 2009). Bees with longer glossae are thought to have more specialized diets, leaving them more susceptible to changes in floral habitats that accompany land-use changes (Goulson, *et al.* 2005).

The two long-glossa species in Arkansas are the somewhat uncommon *B. auricomus* and the purportedly declining species *B. pensylvanicus.* The only species that has experienced a decline in county-level occurrence in Arkansas is *B. pensylvanicus,* a late-emerging, long-glossa species with a long active period. This supports the hypothesis that the interaction between these factors might predispose some bumble bee species to decline (Williams, *et al.* 2009).

For each species that occurs in Arkansas, I report the local phenology, glossa length and plant preferences in the species accounts that follow. Classifying glossa lengths was deemed necessary in order to match the qualitative designations of glossa length used in other bumble bee literature (*e.g.* Kearns and Thomson 2001; Colla, *et al.* 2011). This is especially important considering that some studies include the length of the prementum in measuring glossa length (Goulson and Darvill 2004), rendering comparisons between absolute measurements incompatible.

The plant preferences listed here can be used as a guide for those interested in increasing bumble bee habitat in the region. For example, I found that the wild indigoes, *Baptisia alba* and *Baptisia bracteata,* are preferred by both *B. auricomus* and *B. pensylvanicus,* the two long-glossa species in the state. No single plant species was preferred by all species, but some were common enough among multiple bumble bee species to be highly recommended. A planting of *Silphium integrifolium* (wholeleaf rosinweed), *Monarda fistulosa* (wild bergamot) and *Teucrium canadense* (Canada germander) should appeal to all six *Bombus* species for which plant preference data were gathered. All but five of the plants most preferred by *Bombus* in Arkansas (*Abelmoschus esculentus* (okra), *Carduus nutans* (nodding plumeless thistle), *Centaurea stoebe* (spotted knapweed), *Vicia sativa* (garden vetch) and *V. villosa* (winter vetch)) are native to the area and could be considered when planning pollinator habitat areas.

F. SPECIES ACCOUNTS

The following accounts provide details for each species that has been recorded in Arkansas. The common names of bumble bees are taken from the Entomological Society of America database of Common Names of Insects and Related Organisms (Accessed May 2014, available at http://www.entsoc.org/pubs/common_names), while those of plants are from the United States Department of Agriculture Plants Database (Accessed August 2014, available at http://plants.usda.gov/java/). Glossa lengths are provided by Medler (1962). Data on periods of adult activity and preferred host plants are from observations in Northwest Arkansas as outlined in the methods section.

Bombus auricomus (Robertson, 1903), black and gold bumble bee

Bombus auricomus was not listed as occurring in Arkansas in Franklin's (1912) account of the bumble bees of the new world, but it was recognized (as *B. nevadensis auricomus*) in seven counties in Chandler and McCoy's (1965) statewide account (Fig.II.D1.A). *Bombus auricomus* and its close relative in the west, *B. nevadensis* Cresson, 1874, are currently thought of as separate species (Scholl, *et al.* 1992; Cameron, *et al.* 2007). *Bombus auricomus* is the longest-glossa bumble bee in the state, but it is an early-emerging species compared to others in the area. It has a relatively short active period and is among the rarer species in the state (8% of *Bombus* specimens in the UAAM collection). In Northwest Arkansas, *B. auricomus* is one of the earliest species to establish colonies, and these colonies are typically completed by early July. In other areas of its range, *B. auricomus* seems to follow a different seasonal schedule. The species is listed as a late-emerging species relative to other species in Ontario (Williams, *et al.* 2014) and a mid-season species in Alberta (Hobbs 1965). In Virginia, males were still actively seeking mates in mid-August (Alcock and Alcock 1983), suggesting that colonies in Virginia persist

much later than they do in Arkansas. The distribution of *B. auricomus* in North America seems to be primarily north of Arkansas. Indeed, the southern half of Arkansas is not included in recent range maps of the species (Colla, *et al.* 2011; Williams, *et al.* 2014), although historic records of its occurrence are known (Fig. II.D.1.A). Although uncommon throughout the state, *B. auricomus* has increased in occurrence between the historic (18%) and contemporary periods (31%, Fig. II.D.3).

Bombus auricomus has garnered some conservation attention of late. Throughout North America, *B. auricomus* persists in less than 50% of its historic range, but its relative abundance appears unchanged (Colla, *et al.* 2012). In Arkansas, the presence of *B. auricomus* in the extreme southwestern region of the state was not confirmed in recent surveys of the Blackland Prairie remnants, prompting some concern for its status in the region (Warriner 2011). However, northern Arkansas falls along the southernmost edge of the distribution of *B. auricomus* (Williams, *et al.* 2014), and its occurrence farther south might be infrequent. In the central portion of its range in Illinois, contemporary surveys show that *B. auricomus* is as widely distributed and abundant today as in the past (Grixti, *et al.* 2009). As with species like *B. fraternus* and *B. variabilis*, the relative rarity of *B. auricomus* in some areas of its distribution renders collection records inconsistent and creates a challenge for comparative studies seeking to establish the conservation status of this species.

Glossa length: Long (7.12 ± 0.39 mm)

Adult active period: Early emerging with a short active period (58 days). Majority: mid-May through early July; Earliest: April 18; Latest: August 11

Preferred plants: *Monarda fistulosa* (wild bergamot), *Baptisia alba* (white wild indigo), *Baptisia bracteata* (longbract wild indigo), *Penstemon digitalis* (foxglove beardtongue), *Pycnanthemum tenuifolium* (narrowleaf mountainmint)

***Bombus bimaculatus* Cresson, 1863, twospotted bumble bee**

Bombus bimaculatus is, along with *B. auricomus,* one of the earliest species to emerge in Arkansas. It also has the shortest adult activity period, with the majority of individuals spotted over a period of only 48 days. In spite of its short active period, the number of counties with records of *B. bimaculatus* increased dramatically from 13% of sampled counties in the historic period to 44% in the contemporary period (Fig. II.D.3). *Bombus bimaculatus* showed a strong preference for non-native vetch species, with 64% of all specimens observed on *Vicia sativa* and *V. villosa*. Vetches have been naturalized through much of southeastern North America and are often grown as forage and cover crops, and for erosion control (Owsley 2011). Perhaps their ability to use novel plant resources has contributed to the increased presence of *B. bimaculatus* in Arkansas, although other studies have also reported recent increases in *B. bimaculatus* throughout its range (Colla and Packer 2008; Cameron, *et al.* 2011; Colla, *et al.* 2012).

Glossa length: Medium (5.65 ± 0.64 mm)

Adult active period: Early emerging with a short active period (48 days). Majority: mid-May through late June; Earliest: April 22; Latest: August 1

Preferred plants: *Vicia villosa* (winter vetch), *Vicia sativa* (garden vetch), *Penstemon digitalis* (foxglove beardtongue), *Teucrium canadense* (Canada germander)

***Bombus fervidus* (Fabricius, 1798), yellow bumble bee**

Franklin (1912) reported *B. fervidus* as absent throughout "the greater part of Arkansas", but, lacking deposited specimens, its presence could not be confirmed by Chandler and McCoy (1965). Although *B. fervidus* has occasionally been reported in the state (Franklin 1912; Warriner 2011), its presence here is dubious. A recent survey of *Bombus* in remnant grasslands throughout the state reported *B. fervidus* in Boone and Franklin Counties in 2003 (Warriner 2011), the first such sightings since it was reported 90 years prior (Franklin 1912). The Boone County specimen was the only state record of this species with a deposited voucher specimen. Another historical specimen identified as *B. fervidus* is among the specimens in the UAAM collection: a male collected October 1, 1963 in Columbia Co. in the southern extreme of the state. These two specimens deposited in the UAAM collection as *B. fervidus* were both males, yet investigations of genitalic characters shows that they are actually *B. pensylvanicus*.

Males of *B. fervidus* superficially resemble some of the variants of male *B. pensylvanicus*, and the two species can be difficult to distinguish (Mitchell 1962). Although Mitchell (1962) suggests a number of external characters that can be helpful in distinguishing the two, male *B. pensylvanicus* and *B. fervidus* can only be reliably distinguished by comparing their genitalia (Fig. II.F.1.A–B). The most obvious difference is in the penis valves (*sensu* Mitchell 1962; Michener 2007). The enlarged apices of the penis valves of *B. pensylvanicus* are long and slender, while the apices of those of *B. fervidus* are more truncate, with the breadth and width about equal. Additionally, the interior process of the gonostylus of *B pensylvanicus* is flattened and broad, unlike that of *B. fervidus*. *Bombus fervidus* was not observed in 2011–2013 standardized surveys that we conducted throughout the northwestern portion of Arkansas, despite intensive sampling each season (number of observations=1,693). The North American

distribution of *B. fervidus* appears to be primarily western and northeastern (Koch, *et al.* 2012; Williams, *et al.* 2014). To date, there are no deposited specimens of *B. fervidus* collected in Arkansas. Although we cannot discount its occasional presence in Arkansas, it seems more likely that literature records of this species in Arkansas are based on misidentifications, rather than true occurrences.

Glossa length: Long (6.50 ± 0.74 mm)

Adult active period: Not in the state

Preferred plants: Unknown

***Bombus fraternus* Smith, 1854, southern plains bumble bee**

In their museum survey, Chandler and McCoy (1965) noted *B. fraternus* as "widespread", and it was recorded in as many counties as *B. griseocollis* (Fig. II.D.2.C–D). *Bombus fraternus* remains widely distributed across Arkansas, and its occurrence has remained stable between the historic (36%) and contemporary periods (33%, Fig. II.D.3). Although *B. fraternus* appears to have a wide geographic distribution, it is relatively less abundant than its congeners (Grixti, *et al.* 2009; Colla, *et al.* 2012). There are some indications that *B. fraternus* might be declining, but its relative rarity makes it difficult to be certain of its status. Throughout its range, *B. fraternus* has declined in relative abundance and in geographic persistence, but its relative abundance over all museum records was only 0.32% (Colla, *et al.* 2012). Similarly, an Illinois study designated *B. fraternus* as declining after finding that it was absent from the southern region of the state where it was formerly present, but its relative abundance ranged from 0.2–1.9% over all studied records spanning 1900 to 2007 (Grixti, *et al.* 2009).

Glossa length: Short (4.69 ± 0.37 mm)

Adult active period: Late emerging with a long active period (92 days). Majority: early July through early October; Earliest: April 6; Latest: October 3

Preferred plants*: Passiflora incarnata* (purple passionflower), *Silphium integrifolium* (wholeleaf rosinweed), *Solidago* (goldenrod), *Liatris pycnostachya* (prairie blazing star), *Silphium* (rosinweed), *Bidens aristosa* (bearded beggarticks), *Cephalanthus occidentalis* (common buttonbush), *Solidago altissima* (Canada goldenrod), *Verbesina virginica* (white crownbeard)

***Bombus griseocollis* (DeGeer, 1773), brownbelted bumble bee**

Bombus griseocollis is a widely distributed species in both eastern and western North America (Koch, *et al.* 2012; Williams, *et al.* 2014). Although the species might be declining in the northeastern portion of its range (Williams, *et al.* 2014), the occurrence of *B. griseocollis* has greatly increased between the historic (36%) and contemporary periods (56%, Fig. II.D.3) within Arkansas. Two specimens in UAMM were captured in the period between the sampling periods in this study (1966–1999): Johnson Co., July, 1978 and Cleburne Co., April 19, 1969.

Glossa length: Short (4.91 ± 0.50 mm)

Adult active period: Early emerging with a short active period (60 days). Majority: early June through early August; Earliest: April 18; Latest: October 15

Preferred plants*: Cephalanthus occidentalis* (common buttonbush), *Pycnanthemum tenuifolium* (narrowleaf mountainmint), *Teucrium canadense* (Canada germander), *Liatris pycnostachya* (prairie blazing star), *Carduus nutans* (nodding plumeless thistle), *Asclepias hirtella* (green milkweed), *Asclepias viridis* (green antelopehorn), *Vicia villosa* (winter vetch), *Centaurea*

stoebe (spotted knapweed), *Monarda fistulosa* (wild bergamot), *Silphium integrifolium* (wholeleaf rosinweed)

Bombus impatiens Cresson, 1863, common eastern bumble bee

The occurrence of *B. impatiens* has more than tripled between the historic (21%) and contemporary sample periods (72%, Fig. II.D.3). This is consistent with other reports of *B. impatiens* throughout its range (Colla and Packer 2008; Cameron, *et al.* 2011; Colla, *et al.* 2012). The UAAM collection holds two specimens collected between the historical and contemporary periods: Polk Co., June 4, 1963 and Saline Co., August 17, 1976. In the United States, *B. impatiens* is the only bumble bee species currently mass-reared for pollination services and has been commercially available since 1990 (Velthuis and van Doorn 2006). The ecological repercussions of commercial bumble bee trafficking are largely unknown. The greatest concern has been the potential for pathogen spillover, the transmission of diseases from commercial colonies to wild ones. Commercial bumble bee colonies are known to support heavier loads of pathogens, such as the intestinal protozoa *Crithidia bombi* Gorunov, 1987 and *Nosema bombi* Fantham and Porter, 1914, and parasites, such as the tracheal mite *Locustacarus buchneri* (Stammer, 1951), than their wild counterparts. Wild bees foraging near greenhouses in Canada which utilize commercial bumble bees are more likely to be infected with *C. bombi* and *N. bombi* than wild bees located far from greenhouses (Colla, *et al.* 2006). This pathogen spillover from commercial bumble bees to wild populations could pose a threat to the stability of wild bumble bee populations. The commercial use of *B. impatiens* might also have another potential ecological impact that has remained unexplored: artificially increasing the local abundance of the commercial species through augmentation. If this were the case, we might expect *B. impatiens* to be less common in wildlands than in areas near agricultural development. Indeed, *B. impatiens*

was rarely encountered in surveys of Arkansas grasslands from 2002 to 2008 (Warriner 2011), in spite of its recent increase in county-level records. Whether or not the commercial trafficking of *B. impatiens* has influenced localized increases in Arkansas and elsewhere is unknown, but it is a notion that warrants further study.

Glossa length: Short (4.74 ± 0.62 mm)

Adult active period: Late emerging with an intermediate active period (75 days). Majority: mid-July through early October; Earliest: April 22; Latest: October 20

Preferred plants*: Solidago speciosa* (showy goldenrod), *Symphyotrichum* (aster), *Silphium integrifolium* (wholeleaf rosinweed), *Solidago* (goldenrod), *Pycnanthemum pilosum* (whorled mountainmint), *Verbesina alternifolia* (wingstem), *Verbesina virginica* (white crownbeard), *Solidago altissima* (Canada goldenrod), *Salvia azurea* (azure blue sage)

***Bombus pensylvanicus* (DeGeer, 1773), American bumble bee**

Bombus pensylvanicus (as *B. americanorum* (Fabricius, 1775)) was listed as the "most widespread and common species" in the state in Chandler and McCoy's (1965) study. Its state-wide occurrence is much reduced today, although it remains present throughout the state (Fig. II.D.1.F). The contemporary occurrence of *B. pensylvanicus* (50% of sampled counties) is 61% of its occurrence in the historic period (82%, Fig. II.D.3). Only a single record occurred in the period between our sampling intervals: Faulkner Co., September 6, 1976. This state-level pattern reflects what has also been observed throughout the range of *B. pensylvanicus*, and many sources consider *B. pensylvanicus* to be a declining species (Colla and Packer 2008; Grixti, *et al.* 2009; Cameron, *et al.* 2011; Colla, *et al.* 2012). Although there are indications of a range-wide decline of *B. pensylvanicus*, it is likely that not all areas are reflecting the same shifts in abundance or

occurrence. For example, *B. pensylvanicus* was abundant in the extreme south and western portions of its range (Louisiana, Oklahoma and Texas) in recent surveys, although it was absent from much of the north and eastern areas in which it was expected to occur (Cameron, *et al.* 2011). Similarly, *B. pensylvanicus* was absent from the northern region of Illinois in recent surveys, although it was known from northern Illinois in historical records (Grixti, *et al.* 2009). This heterogeneity highlights the utility of localized studies in determining the conservation status of species of interest.

Glossa length: Long (6.41 ± 0.58 mm)

Adult active period: Late emerging with a long active period (82 days). Majority: late June through mid-September; Earliest: May 13; Latest: October 16

Preferred plants*: Baptisia alba* (wild white indigo), *Vernonia* (ironweed) , *Teucrium canadense* (Canada germander), *Monarda fistulosa* (wild bergamot), *Abelmoschus esculentus* (okra), *Solanum carolinense* (Carolina horsenettle), *Cirsium discolor* (field thistle), *Salvia azurea* (azure blue sage), *Silphium integrifolium* (wholeleaf rosinweed), *Vicia villosa* (winter vetch)

***Bombus variabilis* (Cresson, 1872), variable cuckoo bumble bee**

Prior to this examination, only a single record of this species in Arkansas existed in the literature. Chandler and McCoy (1965) listed a single record from Washington County, but without including any additional collection information. Three specimens of *B. variablis* collected in Washington Co. during our target historical period were among the specimens in the UAAM collection (September-1900, August 15, 1906 and October 1, 1961), yet no specimens for the contemporary period were present (Fig. II.D.1.G). However, three additional male specimens that were collected outside of our historical and contemporary periods are present in

UAAM. Two specimens were collected in the northwest portion of the state (Franklin Co., October 5, 1976 and Washington Co., September 29, 1993); the other was collected in eastern Arkansas (Desha Co., August 7, 1966). *Bombus variabilis* was not recovered in this survey nor in Warriner's (2011) extensive Arkansas grassland surveys. With so few records, there is no suggestion of a change in the occurrence of *B. variabilis* between the historic (2.6%) and contemporary periods (0%, Fig. II.D.3).

Records for this species are both temporally and spatially sporadic throughout eastern North America (Williams, *et al.* 2014). The species is a member of the cleptoparasitic subgenus *Psithyrus* whose host is *B. pensylvanicus.* Its unusual life history might help explain its rarity. Lacking a foraging worker caste, *Psithyrus* bumble bees are nest-bound and less likely to be encountered in typical field surveys. Also, as obligate nest parasites, their abundance is bound to be lower than that of their host. Still, there are indications that *B. variabilis* is declining and deserves further study. Its host, *B. pensylvanicus,* is also suspected to be on the decline (Cameron, *et al.* 2011; Colla, *et al.* 2012), and an obligate parasite is likely to follow the same population trends as its host. Across its range, *B. variabilis* has dramatically declined both in abundance relative to other *Bombus* species and in geographic persistence, leading to a recommendation that it be classified as "critically endangered" (Colla, *et al.* 2012). As in the case of *B. fraternus,* I urge that studies aiming to determine the conservation status of this rarer species take into consideration the inherent difficulties in accurately sampling species with low detectability before drawing conclusions on its stability.

Glossa length: Unknown, Not reported

Adult active period: Unknown, Records in Arkansas from August - September

Preferred plants: Unknown, Not observed

G. Acknowledgements

I would like to thank Jeffery Barnes (UAAM) for assistance and access to the collection, Clinton Trammel for specimens and photograph, as well as Barbara Lewis, Michael Warriner and volunteers throughout the state for providing additional specimens. I also wish to acknowledge the Arkansas Natural Heritage Commission for allowing access to state-wide prairie sites (permit numbers S-NHCC-12-013 and S-NHCC-13-010) and Bob Caulk and the Fayetteville Natural Heritage Association for access to prairie restoration sites in the city of Fayetteville, Arkansas.

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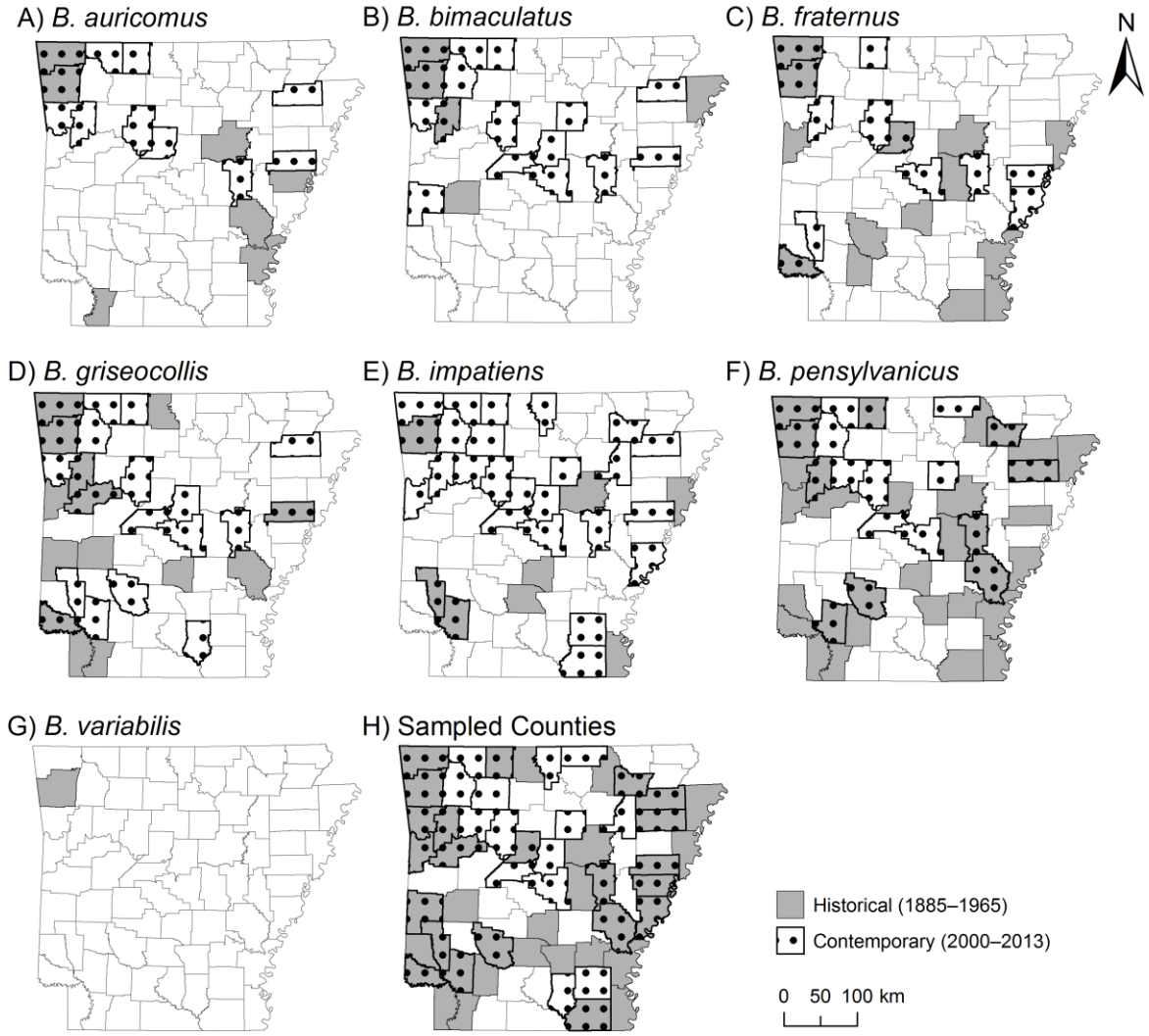

Figure II.D.1.A–H. Maps of Arkansas showing historical (grey) and contemporary (dots) county-level records for each species, and a summary of all records. A) *B. auricomus*, B) *B. bimaculatus*, C) *B. fraternus*, D) *B. griseocollis*, E) *B. impatiens*, F) *B. pensylvanicus*, G) *B. variabilis*, H) Summary of all sampled counties for each period.

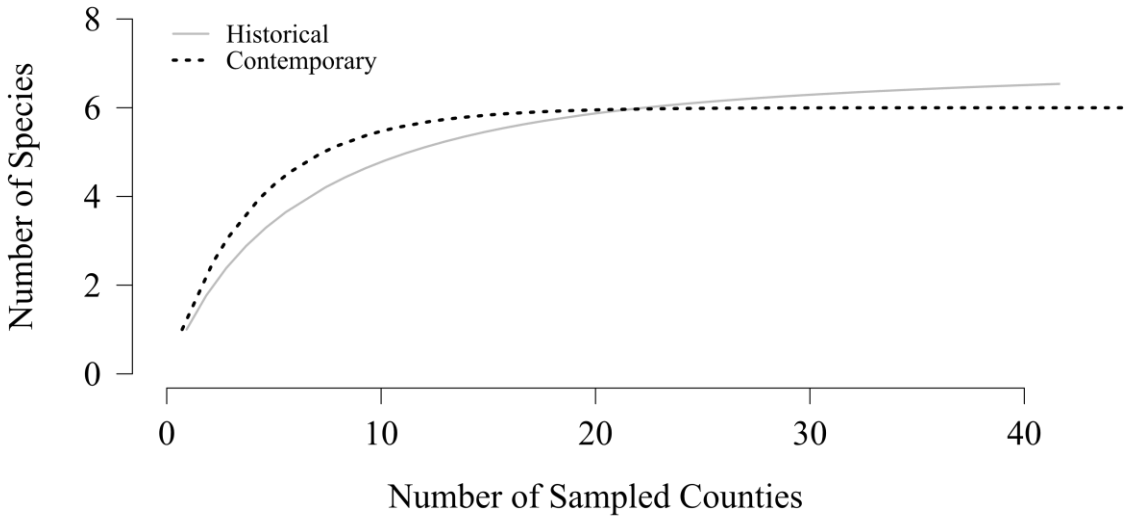

Figure II.D.2. Species accumulation curves for each sampling period. Rarefied accumulation curves were calculated with each county serving as a single sample. Solid grey line = historical period, dashed black line = contemporary period.

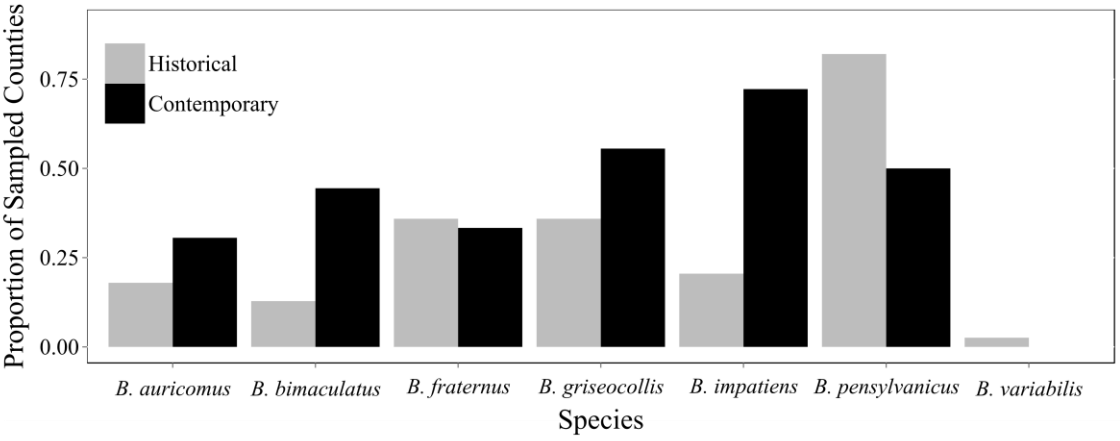

Figure II.D.3. Proportions of sampled counties with records of each bumble bee species in the historical (grey) and contemporary (black) periods in Arkansas.

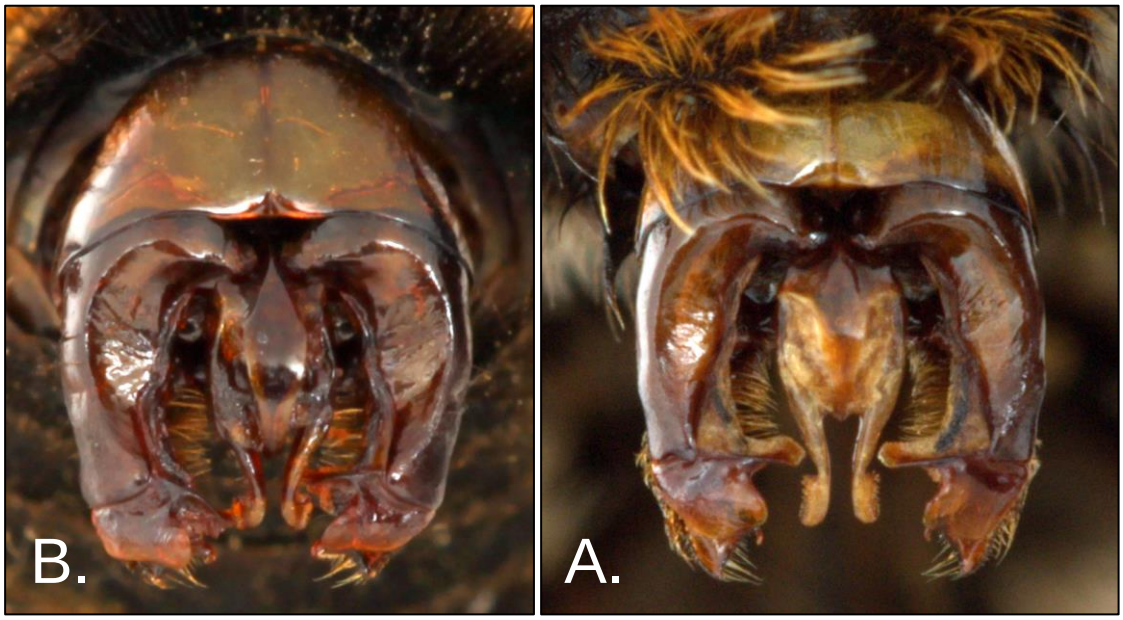

Figure II.F.1.A–B. Male genitalia of A) *Bombus fervidus* and B) *B. pensylvanicus.* A) 15-Sept-1961, Lafayette Indiana; B) 2-July-2003, Boone county, Arkansas. Photographs by Clinton Trammel, used with permission.

III. A MATTER OF SCALE: USING POPULATION GENETICS TO ASSESS THE CONSERVATION STATUS OF BUMBLE BEE POPULATIONS

A. ABSTRACT

Declining bumble bee species have lower genetic diversity than their stable counterparts when compared on a range-wide scale. However, conservation monitoring and management often takes place on a more localized scale, and the utility of microsatellite-based assessments has been untested at this level. The genetic diversity of six species was characterized in northern Arkansas and western Tennessee (*Bombus auricomus, B. bimaculatus, B. fraternus, B. griseocollis* and *B. impatiens,* and *B. pensylvanicus*) with seven to fifteen microsatellite loci. All six species exhibited similar levels of genetic diversity (range of H_S=0.46–0.63), including *B. pensylvanicus,* which was expected to exhibit lower diversity characteristic of its range-wide decline. These results suggest that nationally recommended tools for assessing the conservation status of bumble bees in North America are not applicable at a more localized level, at least in the region studied. This could be an indication of broad stability of these taxa in the region, or it could indicate that recommended microsatellite-based tools are less likely to detect genetic signatures of declining populations at this geographic level.

B. INTRODUCTION

Some bumble bee species are declining in North America, and national surveys show that declining species have low genetic diversity as compared to stable species (Cameron, *et al.* 2011b; Lozier, *et al.* 2011). Thus far, genetic investigations into the stability of *Bombus* Latreille, 1802 (Hymenoptera: Apidae) in North America have been restricted to eight model species, the stable species being *Bombus bimaculatus* Cresson, 1863 and *B. impatiens* Cresson, 1863 in eastern North America and *B. bifarius* Cresson, 1878 and *B. vosnesenskii* Radoszkowski, 1862 in

western North America (Cameron, *et al.* 2011b; Lozier, *et al.* 2011). The declining species are *B. occidentalis* Greene, 1858 in the West and *B. affinis* Cresson, 1863, *B. terricola* Kirby, 1837 and *B. pensylvanicus* (DeGeer, 1773) in the East. These microsatellite (msat)-based investigations of model bumble bee species throughout their ranges have found that the genetic diversity of stable species is higher than that of declining ones, and the correlation between lower genetic diversity and decline has been viewed as a useful monitoring tool to identify species of concern throughout their ranges (Cameron, *et al.* 2011b; Lozier, *et al.* 2011). The development of standardized msat protocols for assessing *Bombus* population structure and genetic variation is a primary goal of the International Union for Conservation of Nature North American Bumble Bee Species Conservation Strategy Genetic and Demographic Issues in Conservation Strategies Working Group (Cameron, *et al.* 2011a). These nationally recommended tools are based upon sampling throughout the range of species, yet conservation management is often applied on a more localized scale such as state, city or county levels (Theobald, *et al.* 2005). Because conservation assessments and monitoring are often conducted on these census scales, it is important to determine if recommended tools can be applied at these levels.

Arkansas museum records mirror the range-wide trends seen in model species (see Chapter II). The number of counties with contemporary records of the stable species *B. bimaculatus* and *B. impatiens* has more than tripled over historical levels, whereas county records of the declining species *B. pensylvanicus* have been reduced to 60% of historical levels. Records of *B. auricomus* (Robertson, 1903), *B. griseocollis* (DeGeer, 1773) and *B. fraternus* (Smith, 1854) in Arkansas did not change between historical and contemporary periods. Analysis of contemporary and historical museum records range-wide indicates that *B. auricomus* and *B. fraternus* might be declining (Colla, *et al.* 2012), although this was not indicated in Arkansas

records. There are some reports that *B. griseocollis* is declining in the northeastern portion of its range (Williams, *et al.* 2014), but range-wide assessments of museum records indicate that it is stable over much of the East (Colla, *et al.* 2012), or increasing as was the case in Arkansas. To date, no genetic assessments have been conducted on the non-model Arkansas species of bumble bees.

This work aimed to test the applicability of nationally recommended population genetic tools on a regional, rather than range-wide, scale in the eastern United States. The genetic diversity and population structure of species known to be stable (*B. bimaculatus* and *B. impatiens*) or declining (*B. pensylvanicus*) were characterized then compared to one another as well as to other species that have not been genetically characterized (*B. auricomus, B. fraternus* and *B. griseocollis*). If these tools are applicable on a regional level, *B. bimaculatus* and *B. impatiens* from northern Arkansas and western Tennessee should exhibit higher diversity than *B. pensylvanicus*. Additionally, comparison with these model species might allow an initial conservation assessment of unexplored species. In keeping with previous findings, I hypothesized that the nationally declining *B. pensylvanicus* would exhibit lower genetic diversity in this region than the nationally stable species *B. bimaculatus* and *B. impatiens.*

C. Materials and Methods

Sampling and Population Designations

Female specimens were collected from 2010 to 2013 from a total of 95 sites in northern Arkansas and western Tennessee using an aerial net and either pinned or stored in 95% ethanol until DNA extraction. Identifications were made using the keys and descriptions of Mitchell (1962). Populations were designated by geo-referencing collection data and inputting coordinates

into ARCGIS v.10.1 (ESRI, Redlands, CA). Buffers were created around each site coordinate with a radius of 1 km, and all sample sites with overlapping radii were considered a single sub-population. Although foraging distances vary among species and have been largely uncharacterized within *Bombus,* workers of most species are thought to forage at distances of less than 1000 m on average (*e.g. B. lapiadarius* (Linnaeus, 1758) 631 m, *B. pascuorum* (Scopoli, 1763) 513 m, *B. terrestris* (Linnaeus, 1758) 267 m (Wolf and Moritz 2008; Carvell, *et al.* 2011), thus for the most part, the buffer distance selected here should be sufficient to capture independently foraging sub-populations. Subpopulations were grouped into two population groups *a priori*, one on each side of the Mississippi River: Arkansas and Tennessee (Fig. III.C.1).

DNA Extraction and Amplification of msat Loci

Total genomic DNA was extracted from either a single mid-leg or one-half of a thorax from individual specimens using a salting-out procedure with in-house reagents (Sambrook and Russell 2001). Briefly, each sample was macerated in 300 µl cell lysis solution and incubated at 80°C for 5 min. One hundred µl of protein precipitate solution were then added; the sample was mixed and centrifuged for 3 min at 13.2 X 1000 rpm. The supernatant was removed and mixed with 300 µl of chilled (-20°C) 100% isopropanol. The sample was then centrifuged for 4 min at 13.2 X 1000 rpm, and the supernatant was discarded. The resulting pellet was then rinsed with 300 µl chilled 70% ethanol and again spun in a centrifuge for 4 min at 13.2 X 1000 rpm. This supernatant was also discarded, and the pellet was allowed to dry at 65°C for 15 min. Finally, the pellet was rehydrated with 50 µl Tris-HCl solution (pH 8.0) and left at ambient temperature overnight. All extractions were subsequently stored at -20°C until use.

Seventeen msat loci were amplified using multiplexes of fluorescently-labeled primer sets (dye set G5, Applied BioSystems, Life Technologies, Grand Island, NY) derived from studies on other *Bombus* species (primers listed in Appendix III.H.1) (Estoup, *et al.* 1995; Estoup, *et al.* 1996; Reber Funk, *et al.* 2006; Stolle, *et al.* 2009). For each sample, 1 µl to 2 µl of extracted DNA were used. The PCR reaction mix included 2.5 µl 5x GoTaq® reaction buffer (Promega, Madison, WI), 0.7 µl 25 mM magnesium chloride, 0.75 µl 200µM each of dNTPs, 0.25 µl of 1 µM BSA, 0.1 µl *Taq* polymerase, 0.1 µl to 0.5 µl of each primer at 20 µM and ultrapure water to make a final volume of 12.5 µl. Thermal cycler settings included a hot start at 95°C, with a 5 min initial denaturation step at 95°C, followed by 30 cycles of 95°C for 30 s, annealing at 55°C or 58°C for 55 s, then 72°C for 30 s, with a final extension step at 72°C for 15 min. Reactions were genotyped with GeneScan 500 LIZ dye size standard (Applied Biosystems, Life Technologies, Grand Island, NY) on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Life Technologies, Grand Island, NY) at either the Utah State Center for Integrated BioSystems core facility (Logan, Utah) or at the Iowa State University DNA Facility (Ames, Iowa).

Data Analysis

Alleles were scored using the microsatellite plugin v.1.4 available in GENEIOUS v.6.1.6 (Kearse, *et al.* 2012). Samples in which >50% of loci were unamplified or not capable of being scored were removed from the dataset prior to other analyses. Likewise, loci which were unamplified or not capable of being scored reliably within >80% of individuals within a species were also excised from the dataset of that species. The resulting allele calls were analyzed with MICROCHECKER v.2.2.3 (van Oosterhout, *et al.* 2004) to examine loci for stutter, null or dropped alleles. Subpopulations were examined for the occurrence of siblings using COLONY

v.2.0.5.0 (Jones and Wang 2010), and one member of each set (same subpopulation, same year) with a > 95% chance of full-sibling status was randomly selected (via highest-number designation with a random number generator in Microsoft Excel) and retained; the remaining suspected siblings were removed from the dataset. As a rough estimate of site-specific diversity, the results of the COLONY analysis were also used to estimate the number of colonies of each species at each site for subpopulations that had more than one sampled individual of a species. Both sub-populations and loci were analyzed for departure from Hardy-Weinberg equilibrium and evidence of linkage disequilibrium among loci using GENEPOP v.4.2.2 (Rousset 2008). Simple Bonferroni corrections for multiple tests were applied to adjust the threshold of significance (α=0.05 / number of loci tested for each species).

Estimates of diversity (H_O=observed heterozygosity, ± the standard error as calculated across subpopulations and H_S = Nei's (1987) genetic diversity) were calculated within each sub-population, population and over all of the data for each species were calculated using the package *hierfstat* (Goudet 2005) within R (R Core Team 2014). The effective number of alleles (A_E), which takes allele frequencies into account, was calculated in *hierfstat.* Differentiation among subpopulations within each population and overall was estimated with Jost's *D* (Jost 2008) using *hierfstat*. Differentiation between *a priori* population assignments (Arkansas and Tennessee) were tested with Analysis of MOlecular VAriance (AMOVA) using *F*-statistics within ARLEQUIN v.3.5.1.2 (Excoffier and Lischer 2010) Bayesian *a posteriori* population assignments were conducted using the program STRUCTURE v.2.3.4 (Pritchard, *et al.* 2000) using a burn-in of 20,000 followed by 100,000 samples and testing K=2–4 clusters for each species.

Pairwise comparisons of genetic diversity (H_S) were conducted in order to test whether or not range-wide tools can be applied at this spatial scale. Specifically, the genetic diversity of stable model species (*B. bimaculatus* and *B. impatiens*) was compared to the genetic diversity of the model declining species (*B. pensylvanicus*), with the expectation that genetic diversity would be lower in the declining species (one-tailed Mann-Whitney U-tests). Non-model species were also compared to model species, but without expectations of which might be higher or lower (two-tailed Mann-Whitney U-tests). Because estimates of diversity can be inaccurate with low sample sizes, only species with 25 or more samples were compared (Hale, *et al.* 2012), excluding *B. fraternus* from pairwise comparisons. Because microsatellite loci evolve quickly and their underlying evolutionary histories are largely unknown (Selkoe and Toonen 2006), comparisons were conducted only with subsets of loci shared by both species, and only if five or more loci were shared between species.

D. RESULTS

A total of 483 specimens (366 from Arkansas; 117 from Tennessee) were subjected to analysis (Appendix III.H.2). With the exception of *B. fraternus,* all targeted species were collected in both populations. Not all of the msat loci amplified reliably across all species, and there was evidence for deviation from Hardy-Weinberg Equilibrium and null alleles at some loci in some species (summarized in Table III.D.1). No loci exhibited evidence for linkage disequilibrium. After removing problematic loci, there were seven loci remaining for *B. auricomus, B. bimaculatus* and *B. griseocollis*; eleven for *B. impatiens;* thirteen for *B. pensylvanicus* and fifteen for *B. fraternus.* The occurrence of siblings varied among species (n removed at a ≥95% chance of being full sisters: *B. auricomus* n=9, *B. bimaculatus* n=7, *B. fraternus* n=0, *B. griseocollis* n=4, *B. impatiens* n=13, *B. pensylvanicus* n=21). There were

differences among species in the estimated number of unique colonies sampled at each site. *Bombus griseocollis* had the highest estimate, with an average of 7.4 colonies per site, followed by 5.7 *B. auricomus* colonies per site, 5.1 *B. bimaculatus* colonies per site, 4.2 *B. impatiens* colonies per site, 3.7 *B. pensylvanicus* colonies per site and 1 *B. fraternus* colony per site. A small number of subpopulations in three species showed evidence of departure from Hardy-Weinberg equilibrium (*B. bimaculatus*: 47ARWash (n=13) and 50ARWash (n=3); *B. griseocollis*: 31TNHayw (n=14), 43ARWash (n=6) and 47ARWash (n=5); *B. impatiens*: 44ARWash (n=13)), and these populations were excluded from analysis.

Estimated diversity over all loci retained for each species was similar among species in both populations and overall (Table III.D.2). Genetic diversity (as estimated by Nei's (1987) H_S) ranged from 0.46 (*B. griseocollis*) to 0.63 (*B. bimaculatus*), with overlapping standard errors among all species and population groupings. Estimates of differentiation (as calculated with Jost's (2008) D_{EST} were low among all groups, ranging from zero (*B. griseocollis, B. impatiens* and *B. pensylvanicus*) to a high of 0.00235 (both populations of *B. auricomus*). As such, there was no evidence of population structuring in these groups. Results of the AMOVA analyses concurred, with individuals accounting for the largest proportion of variance in all species (95–100% in all six species, data not shown) and no structure between populations designated *a priori*. There was no evidence of cryptic population structure evident in population assignment tests either, with no clustering in tests with two to four groupings for each species (data not shown).

Pairwise tests of differences in genetic diversity among species were conducted for all possible pairs, with the exception of those involving *B. fraternus*, which had a low sample size, and between *B. auricomus* and *B. griseocollis,* a pair that only shared three loci. In no case was a

difference in diversity found between any species pair (all $p>0.05$, Table III.D.3). Estimates of genetic diversity were found to be equivalent among model species, whether stable (*B. bimaculatus* and *B. impatiens*) or declining (*B. pensylvanicus*), as well as among non-model species (*B. auricomus* and *B. griseocollis*).

E. DISCUSSION

Bombus pensylvanicus is the only species among the six species of *Bombus* in this study region that has shown indications of regional decline in previous studies. Although no studies have been conducted on its status in Tennessee, *B. pensylvanicus* shows a state-wide reduction in county-level records in Arkansas since 2000 as compared to records prior to 1965 (Chapter II). This concurs with other regional and range-wide assessments of this species, and the species is generally considered to be in decline (Colla and Packer 2008; Grixti, *et al.* 2009; Cameron, *et al.* 2011b; Lozier, *et al.* 2011). Because of this, the genetic diversity of *B. pensylvanicus* was expected to be lower than that of species known to be faring well. Contrary to expectations, the genetic diversity within *B. pensylvanicus* was as high as that of *B. bimaculatus* and *B. impatiens*, species known to be stable. All six of the species in this study show similar levels of genetic diversity.

The sum of evidence presented here suggests that nationally-recommended tools for assessing the conservation status of bumble bees in North America are not applicable at a more localized level, at least in the region studied. This could be an indication of broad stability of these taxa in the region, or it could indicate that recommended msat-based tools are less likely to detect genetic signatures of declining populations at this geographic level. Contrary to findings based on range-wide population genetic surveys using msats, the declining *B. pensylvanicus* is as diverse as its presumed stable congeners *B. bimaculatus* and *B. impatiens* in northern Arkansas

and western Tennessee. Although these findings are at odds with range-wide msat surveys, a recent comparison of the genetic diversity between *B. impatiens* and *B. pensylvanicus* using next-generation restriction site-associated DNA sequencing also found the diversity of these two species to be virtually indistinguishable (Lozier 2014).

The differences between range-wide and local studies of *Bombus* genetic diversity have been hinted at before. The large-scale msat study including *B. pensylvanicus* (Cameron, *et al.* 2011b) did not find significant structuring, whereas a localized study within Illinois did find that contemporary populations of this species were structured (Lozier and Cameron 2009). It is unsurprising that populations of a widely distributed species might vary in their genetic composition throughout their range, as ecological and geographic factors that can influence demography, such as elevation, season length and the presence of immigration barriers, are likely to be heterogeneous across a large landscape. Comparisons among species originating from the same locations in a limited area should control for a number of these factors, and such comparisons have been recommended to help determine *Bombus* population stability and recognize populations at risk (Lozier, *et al.* 2011). However, another potential explanation for the findings here is that the msat loci employed might not fully characterize the genetic diversity of each species. Microsatellites are ideal for population genetic studies, because they have a fast mutation rate capable of addressing evolutionary processes that have occurred in recent (demographic and ecological scale) time, are non-coding and selectively neutral and, in the absence of linkage disequilibrium, multiple loci can represent a random sample of the entire genome of an organism that is suitable for statistical analysis (Selkoe and Toonen 2006). Still, the evolutionary histories of individual loci might not conform to these expectations. For example, the locus BL13 was monomorphic in four of the species examined here and was

expressed as the same allele size in three of these species (Table III.D.1). This could indicate that this locus is evolutionarily constrained by factors not explored in this study (*e.g.* if the locus is adjacent to a conserved region of the containing chromosome).

The scope of this study is limited, both in terms of sample size and genomic coverage, thus it would be premature to conclude that msat analyses of *Bombus* conservation status are not of value at localized scales. These tools have the capacity to identify isolated populations at risk that might be evolutionarily significant units (*sensu* Moritz 1994) worthy of conservation attention, regardless of the results of range-wide assessments for a species as a whole. Such unique populations of *B. impatiens* and *B. pensylvanicus* have been revealed on islands using these same msat tools (Lozier, *et al.* 2011). Rather, this study highlights that scale matters in conservation assessments, and that not all tools are applicable at all scales.

F. ACKNOWLEDGEMENTS

I appreciate the wealth of assistance and advice on developing microsatellite protocols for *Bombus* that Joyce Knoblett, Jon Koch and Jamie Strange provided. I would like to thank Clinton Trammel and Roxane Magnus for specimens and laboratory assistance. Also, I wish to thank Jack Grubaugh, Dawn Hall, Soo-Hoon Kim, Lloyd Tripodi and Barbara Lewis for specimens provided for this work. Lastly, thank you to the Arkansas Natural Heritage Commission for access to prairie sites under permit numbers S-NHCC-12-013 and S-NHCC-13-010 and Bob Caulk and the Fayetteville Natural Heritage Association for access to prairie restoration sites in the city of Fayetteville, Arkansas.

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Table III.D.1. Number of alleles and allelic range of 17 msat loci in six *Bombus* species sampled from Arkansas and Tennessee.

	B. auricomus			*B. bimaculatus*			*B. fraternus*			*B. griseocollis*			*B. impatiens*			*B. pensylvanicus*		
Locus Name	N_a	A_E	Range	N_a	A_E	Range	N_a	A_E	Range	N_a	A_E	Range	N_a	A_E	Range	N_a	A_E	Range
BL15	13	7.8	177-203	a	-	-	13	7.0	177-213	a	-	-	c	-	-	a	-	-
B124	13	4.6	243-281	c	-	-	7	5.6	267-279	a	-	-	25	12.0	225-305	a	-	-
BTern01	a	-	-	12	5.2	103-127	13	9.2	89-119	15	8.2	98-128	15	5.6	122-154	14	3.7	91-117
BT28	1	1.0	63	3	1.2	174-180	2	1.1	174-180	5	1.8	181-214	1	1.0	179	1	1.0	186
BT10	20	13.3	89-129	21	8.5	127-181	3	2.5	137-141	20	13.9	130-172	21	12.7	139-185	15	9.8	113-155
BT30	7	3.7	200-218	4	1.2	180-189	a	-	-	a	-	-	7	1.7	178-199	7	3.8	197-215
B96	a	-	-	a	-	-	5	2.2	222-232	c	-	-	13	5.7	237-261	a	-	-
BTMS0081	a	-	-	a	-	-	1	1.0	299	2	1.0	299-303	4	2.2	287-315	a	-	-
BTMS0066	a	-	-	9	4.1	106-142	4	2.6	129-144	8	3.5	128-152	c	-	-	13	5.6	136-184
BTMS0083	a	-	-	a	-	-	1	1.0	495	a	-	-	c	-	-	4	1.9	262-268
B126	a	-	-	a	-	-	b	-	-	c	-	-	a	-	-	4	2.0	140-148
BTMS0062	a	-	-	c	-	-	10	5.1	252-286	c	-	-	37	26.3	235-329	29	19.7	265-347
BTern02	9	5.0	173-189	c	-	-	11	4.7	148-178	c	-	-	a	-	-	16	9.9	167-199
BTMS0086	b	-	-	a	-	-	3	1.7	281-287	4	1.4	272-284	2	1.0	274-280	1	1.0	284
BL13	1	1	159	10	6.1	152-172	1	1	154	1	1.0	154	9	2.6	160-182	1	1.0	154
BTMS0044	c	-	-	10	4.5	269-278	5	4.1	281	c	-	-	9	2.2	272-281	13	5.2	258-273
BTMS0059	d	-	-	a	-	-	3	2.6	341-349	a	-	-	a	-	-	3	1.0	325-341

Letters in the first column of each species indicate loci removed from analysis for the following reasons: a) unreliable amplification, b) evidence for possible null alleles, c) deviation from Hardy-Weinberg equilibrium expectations, d) unreliable scoring due to amplification stutter. N_a = number of alleles detected, A_E = effective number of alleles, Range = length of scored amplicons in base pairs.

Table III.D.2. Summary of genetic diversity and differentiation estimates for *Bombus* species sampled in Arkansas and Tennessee.

Population	Species	N	N_L	$H_O \pm$ SE	$H_S \pm SE$	D_{EST}
Western Tennessee	*B. auricomus*	9	7	0.61 ± 0.16	0.57 ± 0.15	0.0235
	B. bimaculatus	26	7	0.63 ± 0.12	0.63 ± 0.12	0.0080
	B. fraternus	0	-	-	-	-
	B. griseocollis	15	7	0.46 ± 0.15	0.46 ± 0.15	-0.0052
	B. impatiens	28	11	0.57 ± 0.10	0.61 ± 0.10	-0.0188
	B. pensylvanicus	18	13	0.51 ± 0.10	0.55 ± 0.10	-0.0474
Northern Arkansas	*B. auricomus*	54	7	0.61 ± 0.16	0.57 ± 0.15	0.0235
	B. bimaculatus	44	7	0.63 ± 0.12	0.63 ± 0.12	0.0080
	B. fraternus	15	15	0.51 ± 0.09	0.55 ± 0.09	0.0040
	B. griseocollis	60	7	0.46 ± 0.15	0.46 ± 0.15	-0.0052
	B. impatiens	60	11	0.57 ± 0.10	0.61 ± 0.10	-0.0188
	B. pensylvanicus	48	13	0.51 ± 0.10	0.55 ± 0.11	-0.0474
Overall	*B. auricomus*	63	7	0.62 ± 0.16	0.58 ± 0.16	0.0037
	B. bimaculatus	70	7	0.63 ± 0.13	0.62 ± 0.12	0.0130
	B. fraternus	15	15	0.51 ± 0.09	0.55 ± 0.09	0.0040
	B. griseocollis	75	7	0.46 ± 0.15	0.46 ± 0.15	-0.0155
	B. impatiens	88	11	0.58 ± 0.10	0.60 ± 0.10	0.0022
	B. pensylvanicus	66	13	0.51 ± 0.10	0.54 ± 0.11	-0.0284

N = number of sampled individuals, N_L = number of loci successfully genotyped for that species, H_O = observed heterozygosity ± SE= interlocus Standard Error , H_S = genetic diversity ± SE= Standard Error, D_{EST} = Jost's estimated differentiation index. Negative values for D_{EST} should be interpreted as zero.

Table III.D.3. Pairwise tests of genetic diversity between model and non-model species.

Tested Pair	N_L Shared	H_S ± SE	Test	*U*-Statistic	*P*-value
B. bimaculatus	7	0.62 ± 0.12	BP<BB	26.0	0.60
B. pensylvanicus		0.57 ± 0.15			
B. impatiens	8	0.56 ± 0.13	BP<BI	33.5	0.58
B. pensylvanicus		0.52 ± 0.15			
B. bimaculatus	5	0.56 ± 0.17	BB=BI	14.0	0.84
B. impatiens		0.56 ± 0.17			
B. bimaculatus	4	0.42 ± 0.24	BB=BA	6.0	0.66
B. auricomus		0.50 ± 0.20			
B. impatiens	5	0.47 ± 0.20	BI=BA	11.0	0.83
B. auricomus		0.58 ± 0.18			
B. pensylvanicus	5	0.50 ± 0.21	BP=BA	13.0	1.00
B. auricomus		0.49 ± 0.20			
B. bimaculatus	5	0.58 ± 0.17	BB=BG	12.0	1.00
B. griseocollis		0.69 ± 0.14			
B. impatiens	6	0.50 ± 0.16	BI=BG	15.5	0.75
B. griseocollis		0.42 ± 0.17			
B. pensylvanicus	6	0.41 ± 0.18	BP=BG	22.5	0.51
B. griseocollis		0.52 ± 0.15			

N_L Shared = number of loci shared between members of each pair (identities can be determined from Table III.D.1). H_S = genetic diversity as estimated from the loci included in comparison ± SE = interlocus standard error. Tests = either one-tailed (<) or two-tailed (=) comparison with species abbreviated as BA = *B. auricomus*, BB= *B. bimaculatus*, BG = *B. griseocollis*, BI = *B. impatiens* and BP = *B. pensylvanicus. U*-statistic = Mann-Whitney U-test statistic.

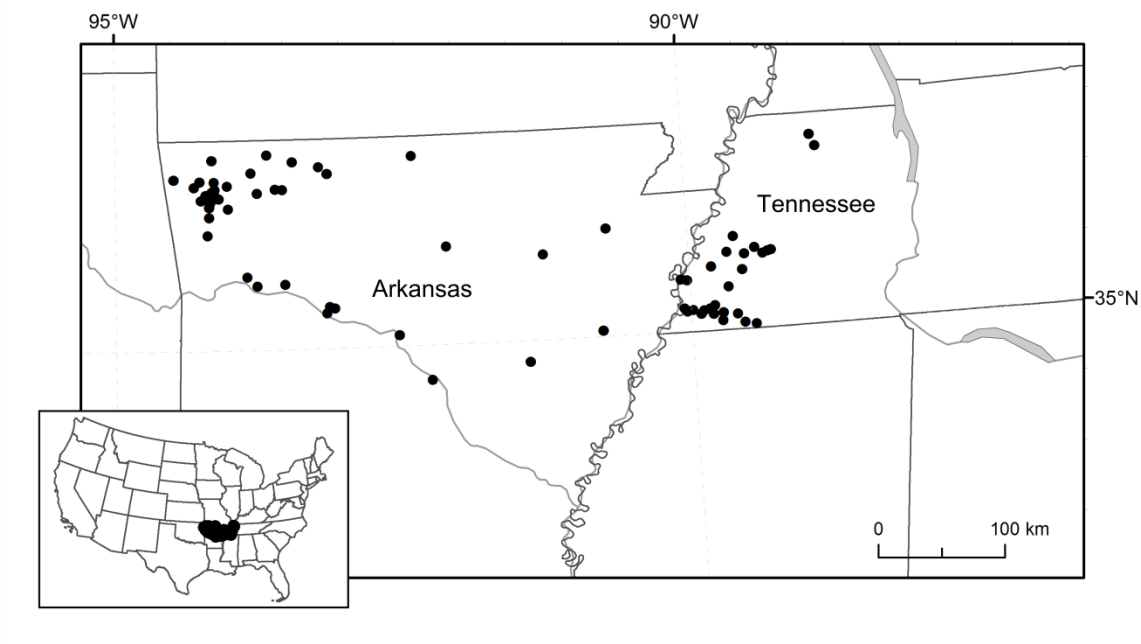

Figure III.C.1. Sample locations in this work and location of the study area (inset). Black dots indicate centroids of sampled subpopulations and are separated by at least 1 km. The two populations Arkansas and Tennessee are bounded by state lines.

Appendix III.H.1. Microsatellite loci, primer sequences, fluorescent tags, annealing temperatures, repeat structure and primer references used in this study.

Locus	Primer Sequences and Tag	T_a	Repeat	Reference
B124	F: *6FAM*-GCAACAGGCGGGTTAGAG R: CAGGATAGGGTAGGTAAGCAG	55	2	A
B126	F: *VIC*-GCTTGCTGGTGAATTGTGC R: CGATTCTCTCGTGTACTCC	58	2	A
B96	F: *PET*-GGAGAGAAAGACCAAG R: GATCGTAATGACTCGATATG	55	2	B
BL13	F: *PET*-CGAATGTTGGGATTTTCGTG R: GCGAGTACGTGTACGTGTTCTATG	58	2	C
BL15	F: *6FAM*-CGAACGAAAACGAAAAAGAGC R: TCTTCTGCTCCTTTCTCCATTC	55	2	C
BT10	F: *NED*-TCTTGCTATCCACCACCCGC R: GGACAGAAGCATAGACGCACCG	55	2	C
BT28	F: *VIC*-TTGCTGACGTTGCTGTGACTGAGG R: TCCTCTGTGTGTTCTCTTACTTGGC	55	3	C
BT30	F: *PET*-ATCGTATTATTGCCACCAACCG R: CAGCAACAGTCACAACAAACGC	55	3	C
Btern01	F: *VIC*-CGTGTTTAGGGTACTGGTGGTC R: GGAGCAAGAGGGCTAGACAAAAG	55	2	C
Btern02	F: *NED*-TTTCCACCCTTCACGCATACAC R: GATTTTATCCTCCGACCGTTCC	58	2	C
BTMS0044	F: *PET*-AGGATCGAGAGAACGAGCTG R: AGGCCTTGGGAGAGTTCG	58	3	D
BTMS0059	F: *PET*-GGCTAGGAAAGATTAGCACTACC R: AGTTCGACAGACCAAGCTGT	58	4	D
BTMS0062	F: *VIC*-CTGTCGCATTATTCGCGGTT R: CTGGGCGTGATTCGATGAAC	58	2	D
BTMS0066	F: *6FAM*-CATGATGACACCACCCAACG R: TTAACGCCCAATGCCTTTCC	58	3	D
BTMS0081	F: *PET*-ACGCGCGCCTTCTACTATC R: AGGGACACGCGAACAGAC	55	4	D
BTMS0083	F: *6FAM*-CGACTCGTTCGAGCGAAATTA R: GTTTTTGCCAGGCTCCGAAT	58	2	D
BTMS0086	F: *NED*-AGAGAAATTGCATGCGGTCG R: CTCGCGCTTGTCGAATCAAT	58	3	D

F: forward primer, R: reverse primer; Fluorescent tags shown in italics; T_a: annealing temperature (°C); References: A: Estoup, *et al.*, 1996; B: Estoup, *et al.*, 1995, C: Reber Funk, *et al.*, 2006; D: Stolle, *et al.*, 2009.

Appendix III.H.2. Samples, sample sites and subpopulations of all specimens used in this study.

						Number of Sampled Individuals of Each Species					
Buffer Subpop	Centroid Latitude	Centroid Longitude	Site Name	Site Latitude	Site Longitude	BA	BB	BF	BG	BI	BP
1	34.748889	-92.269444	1ARPula	34.748889	-92.269444					1	
2	34.845000	-91.415833	2ARPrai	34.845000	-91.415833				1	2	
3	35.023056	-89.441944	3TNFaye	35.023056	-89.441944				1		
4	35.036944	-89.537778	4TNFaye	35.036944	-89.537778				1		
5	35.038370	-90.770938	5ARStFr	35.038370	-90.770938	11	16		4	2	
6	35.059167	-89.730833	6TNShel	35.059167	-89.730833				1		
7	35.076528	-92.540417	7ARFaul	35.075833	-92.536667					1	
			7ARPerr	35.077222	-92.544167					1	1
8	35.099444	-89.597778	8TNFaye	35.099444	-89.597778	1					
9	35.115139	-89.914861	9TNShel	35.114444	-89.904444		1			2	1
			9TNShel2	35.115833	-89.925278		1			2	
10	35.110411	-89.808291	10TNShel	35.101577	-89.808814		2				
			10TNShel2	35.114444	-89.801111		1		2		
			10TNShel3	35.116110	-89.814722					1	
11	35.116944	-89.722222	11TNShel	35.116944	-89.722222		2		2		
12	35.139643	-90.034719	12TNShel	35.139643	-90.034719					1	
13	35.138195	-89.889445	13TNShel	35.135833	-89.879167		1				
			13TNShel2	35.140556	-89.899722					1	
14	35.147222	-89.982778	14TNShel	35.147222	-89.982778		1				
15	35.148472	-89.837639	15TNShel	35.148333	-89.840278				1		
			15TNShel2	35.148611	-89.835000		1		3		
16	35.161389	-90.056389	16TNShel	35.161389	-90.056389		1				
17	35.170000	-89.791111	17TNShel	35.170000	-89.791111						1
18	35.250556	-93.163056	18ARPope	35.250556	-93.163056						1
19	35.282061	-93.094457	19ARPope	35.282061	-93.094457			1			

Appendix III.H.2. (Cont.)

						Number of Sampled Individuals of Each Species					
Buffer Subpop	Centroid Latitude	Centroid Longitude	Site Name	Site Latitude	Site Longitude	BA	BB	BF	BG	BI	BP
20	35.294167	-93.139444	20ARPope	35.294167	-93.139444				1		
21	35.298611	-89.663056	21TNShel	35.298611	-89.663056					1	
22	35.360742	-90.022065	22TNShel	35.358600	-90.019683					1	
			22TNShel2	35.359947	-90.017994					1	
			22TNShel3	35.360853	-90.026944		2				
			22TNShel4	35.362883	-90.019617				1		
23	35.368072	-90.076697	23TNShel	35.368072	-90.076697					1	
24	35.412946	-89.534702	24TNTipt	35.412946	-89.534702	3					1
25	35.448815	-89.806380	25TNTipt	35.448815	-89.806380					4	
26	35.453161	-93.765330	26ARFran	35.453161	-93.765330						1
27	35.462500	-93.523889	27ARJohn	35.462500	-93.523889						1
28	35.520556	-93.852500	28ARFran	35.520556	-93.852500				1		
29	35.521944	-89.350278	29TNHayw	35.521944	-89.350278					2	
30	35.526111	-89.508889	30TNTipt	35.526111	-89.508889		1		1		1
31	35.534947	-89.304332	31TNHayw	35.534947	-89.304332	5	6		14	3	3
32	35.540555	-89.276322	32TNHayw	35.540555	-89.276322		7			4	
33	35.544722	-89.663611	33TNTipt	35.544722	-89.663611				1		
34	35.566389	-89.417778	34TNHayw	35.566389	-89.417778				1	2	
35	35.604671	-91.263435	35ARJack	35.604671	-91.263435					1	
36	35.653889	-89.596111	36TNLaud	35.653889	-89.596111					2	
37	35.693965	-92.108484	37ARCleb	35.693965	-92.108484					1	
38	35.765833	-90.705833	38ARCrai	35.765833	-90.705833	2				1	
39	35.819722	-94.192500	39ARWash	35.819722	-94.192500				1		
40	35.950000	-94.175000	40ARWash	35.950000	-94.175000					2	
41	36.008090	-94.008694	41ARWash	36.008090	-94.008694					1	

Appendix III.H.2. (Cont.)

						Number of Sampled Individuals of Each Species					
Buffer Subpop	Centroid Latitude	Centroid Longitude	Site Name	Site Latitude	Site Longitude	BA	BB	BF	BG	BI	BP
42	36.022347	-94.174228	42ARWash	36.022347	-94.174228		6	1	9		1
43	36.056284	-94.164377	43ARWash	36.051389	-94.164167		1				1
			43ARWash2	36.051894	-94.172728	1	4				7
			43ARWash3	36.061800	-94.160227		3		6	1	
			43ARWash4	36.062670	-94.157342				1	1	
44	36.070272	-94.245192	44ARWash	36.067094	-94.233578	2	1			3	1
			44ARWash2	36.069722	-94.254722					1	
			44ARWash3	36.069744	-94.255090					1	
			44ARWash4	36.070093	-94.255103					6	
			44ARWash5	36.075278	-94.251111		1		1	2	
45	36.081944	-94.196389	45ARWash	36.081944	-94.196389					1	
46	36.082631	-94.088775	46ARWash	36.082631	-94.088775		8		3	2	2
47	36.095603	-94.177405	47ARWash	36.095603	-94.177405	2	13		5	13	2
48	36.108553	-94.204956	48ARWash	36.108553	-94.204956				1		
49	36.116111	-93.752500	49ARMadi	36.116111	-93.752500						3
50	36.118921	-94.130888	50ARWash	36.118921	-94.130888		3			1	
51	36.128181	-94.151544	51ARWash	36.124167	-94.155278	1					
			51ARWash2	36.132194	-94.147811	1	4	1		1	2
52	36.137074	-93.530634	52ARCarr	36.137074	-93.530634	1					
53	36.141389	-93.591667	53ARMadi	36.141389	-93.591667		1				3
54	36.147114	-94.124314	54ARWash	36.147114	-94.124314		1				7
55	36.164540	-94.307181	55ARWash	36.164540	-94.307181			1			3
56	36.171389	-94.013889	56ARWash	36.171389	-94.013889				1		
57	36.200124	-94.128914	57ARWash	36.200124	-94.128914					1	
58	36.205747	-94.256925	58ARWash	36.205747	-94.256925	1	2		1	3	4

Appendix III.H.2. (Cont.)

Buffer Subpop	Centroid Latitude	Centroid Longitude	Site Name	Site Latitude	Site Longitude	Number of Sampled Individuals of Each Species					
						BA	BB	BF	BG	BI	BP
59	36.221636	-94.484357	59ARBent	36.221636	-94.484357			1	11	1	3
60	36.244444	-93.132778	60ARBoon	36.244444	-93.132778	23	1	8	24	26	15
61	36.256133	-88.822883	61TNWeak	36.256133	-88.822883						4
62	36.262923	-93.805523	62ARMadi	36.262923	-93.805523				1	1	
63	36.292778	-93.207500	63ARBoon	36.292778	-93.207500				1		
64	36.335378	-93.436001	64ARCarr	36.335335	-93.435999	1					
			64ARCarr2	36.335421	-93.436002					3	
65	36.340115	-88.867346	65TNWeak	36.337183	-88.868225						4
			65TNWeak2	36.337455	-88.868119						1
			65TNWeak3	36.339147	-88.860092	4					1
			65TNWeak4	36.343083	-88.873803						3
66	36.351799	-92.384148	66ARBaxt	36.351799	-92.384148					1	
67	36.356392	-94.146815	67ARBent	36.356389	-94.149444	3					1
			67ARBent2	36.356395	-94.144186	9	1	2	1	3	7
68	36.386748	-93.659533	68ARCarr	36.385717	-93.658233					1	
			68ARCarr3	36.387778	-93.660833				1		

Buffer Subpop = subpopulations as determined by grouping all sites within a 1 km radius, then determining the coordinates of the centroid of those grouped site locations. Site names include the name of the buffer subpopulation, a two-letter code for population (AR=Arkansas and TN=Tennessee) and the first four letters of the county in which the site was located. Species are listed by the following two-letter codes: BA=*B. auricomus*, BB=*B. bimaculatus*, BF=*B. fraternus*, *BG=B. griseocollis*, BI=*B. impatiens* and BP=*B. pensylvanicus.*

IV. PRELIMINARY SURVEY FOR DIPLOID MALES IN A FEW POPULATIONS OF *BOMBUS* LATREILLE (HYMENOPTERA: APIDAE) USING MICROSATELLITES

A. ABSTRACT

Because sex determination in bumble bees is governed by both haplo-diploidy and complementary sex-determination alleles, the presence of diploid males might indicate inbreeding within populations. Males of five bumble bee species collected throughout Arkansas, Tennessee, Missouri and Mississippi were surveyed for ploidy using seven to fifteen microsatellite loci. Low frequencies of diploid males were found in *B. impatiens* (n=2 out of 7) and *B. bimaculatus* (n=3 out of 41), but not in *B. pensylvanicus* (n=19), *B. fraternus* (n=4) or *B. auricomus* (n=1). Although sample sizes were low, this is the first report of diploid males in the species *B. impatiens* and *B. bimaculatus.*

B. INTRODUCTION

Like most Hymenoptera, sex determination in bumble bees is through haplo-diploidy accompanied by complementary sex determination alleles (Zayed 2009). The haplo-diploid aspect of sex determination dictates that unfertilized eggs will yield males with a single set of chromosomes, and typically, fertilized eggs will yield diploid females with two sets of chromosomes. However, the presence of complementary sex-determination (CSD) alleles can complicate diploid outcomes in some cases. If an individual is diploid and inherits two different CSD alleles, it will develop as a normal, diploid female. On the other hand, if a diploid individual inherits the same CSD allele from both parents ("matched matings" Adams, *et al.* 1977), it will develop as an abnormal diploid male. Although the actual number of CSD loci is unknown in most *Bombus,* single-locus CSD (sl-CSD) is more common among the Hymenoptera and has been experimentally confirmed in two species of *Bombus, B. atratus* Franklin, 1913 and

B. terrestris (Linnaeus, 1758) (reviewed in Harpur, *et al.* 2013). Matched matings can have severe fitness impacts in the case of sl-CSD in social haplo-diploids as each batch of fertilized eggs yields only half the number of workers or queens expected from fertilization. This triggers what has been called the "diploid male vortex", a positive feedback cycle in which the reduced effective population size leads to slower population growth, reduced colony survival and fewer end-of-season reproductives (Zayed and Packer 2005). These characteristics then lead to even smaller effective population sizes and increased inbreeding, and so on, which quickly drives affected populations to extirpation. This suggests that surveys for diploid males can be sensitive indicators of inbreeding and population decline in Hymenoptera, particularly for social species that depend on workers to maximize colony fecundity (Zayed, *et al.* 2004; Zayed 2009).

Although easily conducted using allozymes or microsatellites, diploid male surveys of wild *Bombus* populations are rare, particularly in species thought to be common. In Europe, diploid males have been found at low frequencies in wild populations of two declining species, *B. muscorum* (Linnaeus, 1758) (3 of 64 males, Darvill, *et al.* 2006) and *B. sylvarum* (Linnaeus, 1761) (1 of 39 males, Ellis, *et al.* 2006). A survey of 97 males of *Bombus distinguendus* Morawitz, 1869, a species known to have suffered recent declines in Scotland, found no diploid males (Charman, *et al.* 2010). Similarly, no diploid males were found among isolated populations of the stable species *B. hortorum* (Linnaeus, 1761) in Scotland (0 of 39 males, Goulson, *et al.* 2011). In Japan, diploid males of *B. cryptarum* (Fabricius, 1775) (as *B. florilegus* Panfilov, 1956, (Williams 2010)) a locally rare and threatened species, were found in four of 16 sampled colonies (Takahashi, *et al.* 2008). Two of three species surveyed for diploid males in Alberta, Canada had low frequencies of diploid males: *B. perplexus* Cresson, 1863 (4 out of 104 males), *B. occidentalis* Greene, 1858 (1 out of 112 males) and *B. terricola* Kirby, 1837 (0 out of

81 males) (Whidden and Owen 2011). Of these, *B. occidentalis* is considered to be declining throughout most of its range, with the exception of its northern extreme in Alaska, *B. terricola* is considered to be declining in some parts of its range, but stable in Canada and *B. perplexus* shows no indications of decline (Cameron, *et al.* 2011; Colla, *et al.* 2012; Koch and Strange 2012). These results suggest that diploid males are rare in wild *Bombus* populations and that they occur in both stable and declining species. Although the data are scant, there does not seem to be a consistent pattern of increased frequency of diploid males among declining species relative to stable ones. Clearly, additional surveys including both stable and declining *Bombus* species are warranted.

In eastern North America, *B. bimaculatus* Cresson, 1863 and *B. impatiens* (Cresson, 1863) have been shown to have stable populations throughout their ranges, while *B. pensylvanicus* (DeGeer, 1773) shows strong evidence of recent decline (Cameron, *et al.* 2011; Colla, *et al.* 2012). As part of a broader regional study of genetic diversity in females (chapter III), I surveyed a small number of males of these three species, plus *B. fraternus* (Smith, 1854) and *B. auricomus* (Robertson, 1903), using microsatellites to detect heterozygosity.

C. MATERIALS AND METHODS

Collections were made during 2010–2013 in Arkansas (n=35), Mississippi (n=3), Missouri (n=2) and Tennessee (n=32). Because no structuring has been observed among populations of these species in this region (see Chapter III), all specimens within each species are considered a single, panmictic population here. Male *Bombus* specimens were captured with an aerial net while foraging on flowers, killed in cyanide and preserved in 95% ethanol. Sex was determined by examining the terminalia (females have a stinger, males do not) and counting the number of flagellomeres ($n_{females}$=10, n_{males}=11) and the number of exposed terga ($n_{females}$=6,

n_{males}=7). Species were determined using the keys and descriptions of Mitchell (1962). Specimens have been deposited in the University of Arkansas Arthropod Museum.

DNA extractions were conducted as described in Chapter III.B. Likewise, extractions were subjected to multiplex PCR and sequenced using the same primers and conditions as employed with females (Chapter III, Table III.D.I and Appendix III.H.1) (Estoup, *et al.* 1995; Estoup, *et al.* 1996; Reber Funk, *et al.* 2006; Stolle, *et al.* 2009). Reactions were genotyped on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Life Technologies, Grand Island, NY) at either the Utah State Center for Integrated BioSystems core facility (Logan, Utah) or at the Iowa State University DNA Facility (Ames, Iowa). Alleles were scored using the microsatellite plugin v.1.4 available in GENEIOUS v.6.1.6 (Kearse, *et al.* 2012). Seven to fifteen loci were characterized for each species, with locus selection following the results of quality assessment and Hardy-Weinberg and linkage disequilibrium analysis of females of each species (Table III.D.I). Males that exhibited two alleles at one or more loci were subjected to PCR a second time to confirm diploid status.

The parameter ϕ, an estimate of the proportion of diploids that are male in a population, was calculated for each locus using the following equation, adapted from Owen and Packer (1994):

$$\phi = \frac{\sum B_j}{(1 - \sum p_i^2)T}$$

where B_j = the number of males of that species that have a heterozygous phenotype j, p_i=the frequency of allele i in the sample and T= total number of males of that species characterized at that locus. Although females were also collected from these same sites, this estimator using

male-only data is preferred when population sampling is not assumed to represent real sex ratios (Owen and Packer 1994).

D. Results and Discussion

Seventy-four males were characterized at seven to fifteen microsatellite loci (1 *B. auricomus*, 41 *B. bimaculatus*, 4 *B. fraternus*, 7 *B. impatiens* and 19 *B. pensylvanicus*). Diploid males were present in *B. impatiens* (n=2) and *B. bimaculatus* (n=3), but not in any of the remaining three species. One *B. impatiens* (Shelby County, Tennessee) was heterozygous at four of eleven loci; the other (Washington County, AR) was heterozygous at one locus (Table IV.D.1). Because so few males of this species were examined, no further analysis was conducted, but it is interesting to note that diploid males were detected in such a small sample size. Of the three diploid *B. bimaculatus* males, two (Shelby County, Tennessee and Washington County, Arkansas) were heterozygous at one locus apiece (Table IV.D.2). The third diploid *B. bimaculatus* (Cape Girardeau County, Missouri) was heterozygous at five of seven loci. Estimated frequencies of diploids that are male (ϕ) ranged from 0.030–0.078 (mean 0.042±0.02 SD) across loci.

Colonies that produce diploid males do suffer from a loss of fitness, with slower growth rates and lower colony survival than even other inbred colonies that produce no diploid males, as shown in sibling-mated *B. terrestris* (Whitehorn, *et al.* 2009). However, diploid males are thought to exist at low frequencies (less than about 10%) in most Hymenoptera populations, regardless of the conservation status of the species (Owen and Packer 1994). The estimated frequency of diploid males (ϕ) in this population of *B. bimaculatus* (4.2%) is on par with this estimated baseline and concurs with estimates found in other species using field-caught samples. Canadian populations of *B. perplexus* and *B. occidentalis* had estimated values of ϕ=2.7% and

6%, respectively (Whidden and Owen 2011). About 5% of *B. muscorum* males and 3% of *B. sylvarum* males were diploid in isolated Scottish populations (Darvill, *et al.* 2006; Ellis, *et al.* 2006). In a more thorough study of entire colonies, a Japanese population of *B. cryptarum* had a higher estimated average ϕ of 12.8% (Takahashi, *et al.* 2008).

Although sample sizes in this work were generally too small to make broad generalizations on the actual frequencies of diploid males in these species, this study represents the first report of diploid males in the stable species *B. bimaculatus* and *B. impatiens.* Although diploid male production has been experimentally shown to reduce colony fitness (*e.g.* Whitehorn, *et al.* 2009) and can theoretically increase local extirpation risk in *Bombus* (Zayed 2009), these data show that diploid males can be detected even in small sample sizes of species thought to be stable. This suggests that diploid male detection might not be a simple indicator of population decline. More data on the frequencies of diploid males in wild populations of both stable and declining species are needed in order to develop a conservation assessment tool based on diploid male frequencies in *Bombus*.

E. ACKNOWLEDGMENTS

I would like to thank Joyce Knoblett, Jon Koch and Jamie Strange for assistance with microsatellite protocols and Jack Grubaugh, Joy Layton, Roxane Magnus for additional samples that they provided. I also wish to acknowledge the Arkansas Natural Heritage Commission for allowing access to state-wide prairie sites (permit numbers S-NHCC-12-013 and S-NHCC-13-010) and Bob Caulk and the Fayetteville Natural Heritage Association for access to prairie restoration sites in the city of Fayetteville, Arkansas.

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Table IV.D.1. Number of alleles and heterozygotes at each locus characterized in *B. impatiens* (n=7).

Locus	N_a	N_{het}
B124	2	0
BTern01	3	0
BT28	2	1
BT10	4	0
BT30	2	0
B96	2	1
BTMS0081	3	0
BTMS0062	8	1
BTMS0086	1	0
BL13	5	1
BTMS0044	2	0

N_a = Number of alleles, N_{het} = number of individuals heterozygous at locus.

Table IV.D.2. Number of alleles, heterozygotes and estimated proportion of diploids that are male at each locus in *B. bimaculatus* (n=41).

Locus	N_a	N_{het}	ϕ
BTern01	9	1	0.031
BT28	4	0	0
BT10	10	1	0.030
BT30	2	0	0
BTMS0066	6	2	0.078
BL13	7	1	0.036
BTMS0044	6	1	0.036
	Mean ± SD:		0.042±0.02

N_a = Number of alleles, N_{het} = number of individuals heterozygous at locus, ϕ = estimated proportion of diploids that are male.

V. DISTRIBUTION, DIVERSITY AND ABUNDANCE OF BEES (HYMENOPTERA: APIDAE) ON A REGIONAL SCALE: AUTECOLOGY AND COMMUNITY ECOLOGY OF BEES IN NORTHWEST ARKANSAS

A. ABSTRACT

A bee community consisting of *Apis mellifera*, six species of *Bombus* and *Xylocopa virginica* was characterized over three years of surveys every other week at 13 sites in Northwest Arkansas. This community was active throughout the frost-free growing season in the region (mid-March to late October) and collectively visited 32 families of plants, 88 plant genera and 102 identified plant species, 68% of which were native. All eight bee species were generalists (average diet breadth 10.1±2.08 SD genera), but specialization increased linearly with increasing glossa length (relative maximum dietary preference vs. glossa length, R^2=0.77, p=0.004). The abundance of these bees was positively associated with the richness of flowering plants at surveyed sites, but no measured factors explained site-specific species diversity. Three species, *A. mellifera*, *X. virginica* and *B. griseocollis*, were much more abundant than the other five species and collectively accounted for 86% of observations (n=5,942). The two species with long glossae, *B. auricomus* (6.27±0.57 mm) and *B. pensylvanicus* (6.35±0.98 mm), were more specialized in their floral choices (relative maximum preferences: 27.2 and 18.2, respectively) and showed high niche overlap (O_{12}=0.540, p<0.001), yet their divergent phenologies likely prevent direct competition between these two species. This is a somewhat novel finding that might help explain why bumble bee communities are more speciose than expected under strict competitive exclusion hypotheses. Understanding the factors that drive the local abundance of these important pollinators should provide helpful insights into habitat management for bee conservation in the region.

B. INTRODUCTION

Basic ecology can inform conservation management efforts, yet for many pollinators this information is limited. Bees are often considered as a single guild, in spite of species-specific differences that might influence their responses to management efforts. Characteristics such as glossa length, body size, foraging distance, sociality and active period likely drive differences in the responses of individual pollinator species to local- and landscape- level environmental factors. On the other hand, generalist bee species might overlap in their habitat requirements (*e.g.* floral resources), in which case habitat management efforts could be directed to benefit multiple species. Conservation management requires understanding both the divergent characteristics among species as well as the aspects that unite them as a community.

Bee communities often contain a large number of species (are speciose), but those species are not equally abundant (exhibit low species evenness). In a survey of Alachua County organic farms in north-central Florida, eight species accounted for 88% of the 4,662 bees collected at seven sites (Hall and Ascher 2011). In a survey of seven sites along a 77 km stretch of the Black Belt Prairie in Mississippi, 118 species were identified from 6,138 total specimens collected (Smith, *et al.* 2012). The most commonly collected group was the genus *Lasioglossum* Curtis, 1833 (Hymenoptera: Halictidae), which accounted for 38.5% of all specimens collected. Although smaller, solitary bees (usually Halictidae) often numerically dominate bee diversity studies, some Apidae, including the native *Bombus* Latreille 1802 and exotic *Apis mellifera* Linnaeus 1758, are very common in most bee-visited areas within the eastern United States and could often be dominant pollinators within certain systems. In the Mississippi survey, for example, *Bombus* accounted for 16.8% of all specimens collected (Smith, *et al.* 2012). *Apis mellifera* were not quantified, but the authors state that honey bees were present at all surveyed

sites. A study of bee visitors to experimental native plant plots in Michigan found that, of the 2,221 bees observed, 864 (39%) of these were *A. mellifera* and another 872 (39%) were *Bombus*, most of which were *B. impatiens* Cresson, 1863 (Tuell, *et al.* 2008). Of bees surveyed in blueberry fields throughout the southeastern US, less than 4% were species other than honey bees, bumble bees and the southeastern blueberry bee (*Habropoda laboriosa* (Fabricius, 1804) (Hymenoptera: Apidae)), a *Vaccinium* specialist (Cane and Payne 1993).

Bees within a community are not just coexisting; often they are sharing resources. For example, floral niche overlap by *A. mellifera* and *Bombus* species can be quite high in natural systems. In a California nature reserve, overlap ranged from 40 to 70%, and in a particularly dry year, a single plant species accounted for 88% of honey bee and 61% of bumble bee foraging observations (Thomson 2006). In Arkansas, *Xylocopa virginica* (Linnaeus, 1771) is another common species that shares the same floral resources as *Bombus* and *A. mellifera.* Although published surveys rarely rank *X. virginica* as one of the most common bees observed, it is ubiquitous in Arkansas and often quite abundant. All three groups are generalists and concurrently visit many plant species over long periods of adult activity. Bumble bees are active from March to October in the study region, concurrent with *A. mellifera* and *X. virginica.* Although there are many other bee species within this community, limiting the bee community under consideration to the eight species in the genera *Apis*, *Bombus* and *Xylocopa* allowed each species to be characterized in-depth.

The overarching goal of this work is to characterize the bee community consisting of the members of the genera *Apis*, *Bombus* and *Xylocopa* in Northwest Arkansas in order to provide an ecological foundation that could be of use in regional bee conservation. Prior to this work, no efforts were made to characterize the bee community in the region. Floral resources are a

fundamental element that unites the members of this community and the first objective of this work is to characterize the floral resources used by bees in this community. In addition to providing lists of flowers used by bees that could aid habitat management efforts, I also explore the diet breadth, specialization and the potential for competition among the bee species under investigation here. The second objective is to characterize the phenology of bees in the region, as this could prove informative in efforts to understand community dynamics and potential agricultural application of pollination services, and allow for more accurate species monitoring for conservation efforts. The third objective is to characterize the site-based factors that could be responsible for localized differences in bee communities and to test the influence of these factors on the abundance and diversity of species. Understanding the factors that drive local abundance and diversity should provide helpful insights into habitat management for bee conservation.

In this study, I surveyed 13 sites in Northwest Arkansas every other week for three groups of bees: honey bees (*A. mellifera*), bumble bees (six species) and large carpenter bees (*X. virginica*) over a three-year period, 2011–2013. Plant associations were recorded for two of these years, 2012–2013. I then compared bee abundances and site-based diversity to various site, local and landscape factors in order to determine which factors influence bee communities in the region. I hypothesize that 1) some of these species will overlap in their use of floral resources, while others will occupy more specialized niches, 2) *Bombus* species are temporally specialized, with divergent phenologies and 3) site-based characteristics such as land use and floral diversity help explain both the differential abundance and diversity of bees among sites and that different species will respond to these factors at different spatial scales.

C. MATERIALS AND METHODS

Study Locations and Site Descriptions

Thirteen sites were chosen throughout the Ozark Highlands ecoregion of Northwest Arkansas on the basis of several characteristics, such as *a priori* land-use type, apparent floral resources, size, accessibility, management and land-use stability throughout the course of the study. Based on initial surveys, each site was given a general designation based on apparent land-use type and management regimes. These were dubbed Farms (n=2), Habitats (n=4), Lots (n=4) and Prairies (n=3), as explained below. Locations of the chosen sites and their *a priori* land-use types are shown in Figure V.C.1 and described in Table V.C.1.

The two farms used in this study both produced multiple crops throughout the growing season and were a similar size (Table V.C.1). Dickey Farm (Dickey) was 4.32 ha and located near Tontitown, Arkansas. Horn Farm (Horn) was 4.15 ha and located in Elm Springs, Arkansas. Both farms grew strawberries, tomatoes, peppers and pumpkins. Dickey also grew grapes, apples, plums, lettuce and corn. Horn also grew squash, cucumbers, okra, greens and beans. Although not included in the survey area, Horn also contained areas of pasture reserved for goats and horses. In addition to area devoted to agriculture, both sites included developed areas (*e.g.* buildings, unpaved roads) and undeveloped margins.

The four areas designated as "Habitats" were prairie restoration areas within the Fayetteville, AR city limits. All of these sites are owned by the City of Fayetteville and managed as Wildlife Habitat Areas by the Fayetteville Natural Heritage Association (FHNA). These four sites were chosen by FHNA to represent their conservation efforts throughout the city limits. All four sites have been designated as Certified Wildlife Habitat by the National Wildlife Federation.

The Lake Fayetteville (Lake Fay) site included remnant tallgrass prairie and was actively managed by mechanically removing invasive plants, seeding native grasses and forbs, selective herbicide applications, mowing and controlled burning. Restoration efforts began in 2009 with Unit 1 at the northeastern extreme of the park. Restoration began on Unit 2, an area adjacent to Unit 1, in the following year, with subsequent units added yearly. In this study, only Units 1 and 2 were surveyed for a total survey area of 8.62 ha. Strips and patches of woody plants were present within the surveyed portion, but only the edges of these areas were included in surveys. Although paved trails border much of the survey area, there were no trails within the study area.

Restorations of both the Paul R. Noland Wildlife Habitat (Noland) and the Woolsey Wet Prairie Sanctuary (Woolsey) were mitigation projects initiated to compensate for wetland habitat lost to the construction of city wastewater treatment plants. In 2006, restoration began on the 19.27 ha Woolsey site, formerly a wet, tallgrass prairie. Although the restoration and subsequent management of Woolsey was similar to that of Lake Fayetteville, the native plant seedbank was intact at the site and did not require seeding to restore a native plant community. Also, to maintain the wetland habitat, multiple berms were constructed to augment the existing, natural prairie mounds and provide wet lowland areas throughout the site. Highland areas were mowed and maintained as unpaved trails throughout the site. Mitigation and restoration efforts began at Noland in 2009 and included the same management tactics as Lake Fayetteville. The 2.94 ha site was converted from fescue pasture to a mixed-species prairie and included a 61 m wooded riparian zone. The surveyed area also included a gravel lot and walking trail.

In 2005, the City of Fayetteville purchased World Peace Wetland Prairie (World), a 0.84 ha undeveloped site with a combination of remnant native plants and invasive weeds. Since then, restoration has solely relied upon occasional mechanical removal of invasive plant species. An

unpaved, infrequently mowed trail bisected the survey area, which included woody and shrubby portions. One small area was planted with native and non-native flowering plants and irrigated occasionally, while the majority of the site was left wild.

The four sites designated as "Lots" were undeveloped areas that received little to no management. Two sites are roadside areas along AR-412. Although their land-use histories are uncertain, the Madison County site (Madison) is 0.72 ha along one of Arkansas' Wildflower Routes and likely received seeding treatments from the Arkansas Highway and Transportation Department's Wildflower Program. The Madison site received mowing treatments a few times a year. The Carroll County roadside plot (Carroll) consisted of a 1.26 ha strip of mixed forbs on a sloping hillside. The site received no mowing and was devoid of trees. The Sunrise Mountain plot (Sunrise) was a privately-owned, undeveloped 3.69 ha area of grasses, forbs and Eastern Cedars, with forested borders. Although the land-use history of the site was unknown, there were signs of previous development on-site, including remnants of concrete foundation. No management actions occurred at this site during the course of the study. The Golden site consisted of 1.17 ha of a former home site, abandoned some time ago. The area contained a riparian zone and received infrequent mowing throughout the study duration.

The three "Prairie" sites were restored prairie remnant areas managed by the Arkansas Natural Heritage Commission (ANHC). All Prairie sites were actively managed by ANHC by removing invasive plants, seeding native plants, applying herbicide, mowing and controlled burning. The prairie sites were dominated by native grasses and forbs, although non-native plants were also present. Baker Prairie (Baker) was a 28 ha remnant prairie in Harrison, Arkansas. Formerly used as pastureland, the site has never been plowed and has been under intensive restoration management since 1992 (McKenzie, *et al.* 2006). Baker was bisected by a paved

road, and only the eastern 11.45 ha portion was used in this study. The study site consisted of rolling hills with a small, shrubby riparian area at the base of one hill. A frequently mowed trail transversed the entire site. Searles Prairie (Searles) was a 3.01 ha remnant prairie in Rogers, Arkansas. The site was hay pasture prior to being placed under ANHC management in 1988 (McKenzie, *et al.* 2003). Searles contained an ephemeral pond and an infrequently-mowed trail. Cheney Prairie (Chesney) was a 33.4 ha remnant, tallgrass prairie near Siloam Springs, Arkansas which came under ANHC management in 2000. A 24.31 ha area which included an ephemeral creek and wooded riparian zone was included in the study. Mowed trails encircled and bisected the site. The size of each site ranged from 0.72 to 24.3 ha, with an average area of 6.6 ha. Table V.C.1 gives the site type, latitude-longitude coordinates and characteristics of each site used in this study.

Weather and Degree Day Calculations

In order to assess the influence of temperature on the seasonal progression of bees at these sites, the year-to-year variation in weather throughout the study region was characterized by employing degree-day models. In brief, these models apply a threshold value below which biological development is stunted. For each day that reaches a mean temperature above this threshold, the degrees that are above the threshold are counted and these are summed successively over time. Although lower developmental threshold temperatures are best determined through laboratory development studies that can accurately measure such things for each organism, no such data exist for *Bombus* or *Xylocopa*. I have chosen a lower threshold of 10°C for this study. This is a typical default value when true values are unknown (*e.g.* Petersen, *et al.* 2013). Because this model was intended merely as a means of standardizing the

progression of seasons from year to year, I have chosen the simplest model, a simple average of daily mean temperature minus the lower threshold (T_{base} = 10°C):

$$DD_{10} = \frac{T_{max} + T_{min}}{2} - T_{base}$$

where DD_{10} = degree-day base ten, T_{min} = minimum temperature recorded on a single day and T_{max} = maximum temperature that same day (Pedigo 2002). A value of zero is recorded for days in which the average of the daily mean temperature is less than the lower threshold. The cumulative degrees days were determined through simple summation for each day in the calendar year starting at January 1.

Degree day calculations and daily temperature data for years 2011–2013 were obtained from the University of Oregon's Integrated Plant Protection Center website (http://uspest.org/SC/AR/). Arkansas weather stations that were in closest proximity to sample sites were chosen to provide data for each site (Fig. V.C.2), resulting in data from seven weather stations. The distances between sites and weather stations ranged from 1.2 km (Lake Fayetteville site to Springdale CW3927 station) to 28.6 km (Carroll County site to Eureka Springs DW6195). Data for 2011 were not available from the Eureka Springs DW6195 station, so data from the next closest station, Harrison Co Boone Apt (35.3 km away) were used for the Carroll site in that year. The daily variation in degree day accumulation among stations was used to calculate the standard error for each calendar date. Daily accumulation of precipitation was also gathered from the same data source.

Survey and Sampling Methods

Surveys for *Bombus*, *Xylocopa virginica* and *Apis mellifera* (hereafter, "bees") were usually conducted every other week between 16 May, 2011 and Oct 20, 2013 (2011: 16 May through 15 October; 2012: 24 March through 26 October; 2013: 16 April through 20 October). Surveys were usually conducted at biweekly intervals, although there were some gaps throughout the three-year study period. Each site was surveyed between 9 and 15 times each year. In Northwest Arkansas, bees are active from early spring until late fall (*e.g.* the first observation of targeted bees in Fayetteville, AR in 2011 was a *X. virginica* female spotted on 17 March, personal observation). This correlates well with the frost-free period in the region which typically begins between April 10–20 and ends between October 20–30 (Andersen 2014). Although bees were present in low numbers in the region after the first fall frost, this event was chosen to mark the end of each field season.

Because the chosen sites were heterogeneous with respect to size and available bee habitat, a survey method that could allow for unbiased comparisons of abundance and composition was necessary. Standardization can be accomplished by converting absolute counts to a relative index based on collection effort (Morris 1960). The survey method I employed was a modified version of recommendations by Silveira and Godínez (1996) for standardized bee faunal surveys. Such surveys often employ time, rather than distance or area, as the unit of uniformity among all abundance measures (*e.g.* Cameron, *et al.* 2011; Koch and Strange 2012). Because flower patches are rarely uniform at any site, non-linear transects targeting floral-rich areas allow a measure of standardization over heterogeneous sites (Connop, *et al.* 2010). All surveys were conducted in fair weather between the hours of 09:00–19:00, usually with little cloud cover and at temperatures ranging from 12.2– 38.9°C as determined from local weather

stations accessed just prior to each survey in 2012 and 2013 (Weather Channel App, iPhone, Weather Channel, LLC Atlanta, GA). Each survey covered an area with an approximate radius of 100 m and was timed (usually conducted for 30 min.).

Prior to initiating each sampling event, I informally scouted each site to locate floral resources that would be likely to be utilized by targeted bees. The flowering plant species at each site were noted, and digital photographs (Canon Powershot Elph 300HS, Melville, NY) were taken to aid in subsequent identification of each using local keys (Smith 1994), known distributions (Kartesz and The Biota of North America Program 2013) and a photographic field guide (Kurz 2010). Additional photographic verification of plant identifications and geographic regions of origin were obtained by consulting regional websites (Tenaglia 2007; Hilty 2012). Plants visited by bees during surveys were identified to species whenever possible, although 14% of observations (n=556) were to plants that were not discernable below the level of genus and 0.4% (n=15) were only identifiable to family. Surveys were conducted by walking at a slow, even pace through the patches of flowers present at the site. Over a 30 min. period, all *Bombus, Xylocopa virginica* and *Apis mellifera* observed within approximately 6 m of the observer were netted with an aerial net. The number of individuals, their species identities, castes and floral hosts were recorded. In 2011, floral host data was recorded merely as a list of occurrences during surveys; in 2012 and 2013, floral host data included bee abundances. Floral hosts were simply the flower upon which the bee was observed foraging, and no effort was made to determine if bees were collecting pollen or nectar, and nectar robbers were included in counts. Most individuals were captured, recorded and immediately released, although some (n=778, 13% of total observations 2010-2013) were retained for other projects (*e.g.* Chapters III and IV). All specimens that were retained were stored in 95% ethanol. Specimens that were missing key

morphological characters and could not be reliably identified in the field were also retained for subsequent identification to species using the keys of Mitchell (1962) and Chandler and McCoy, Jr. (1965). Caste determinations of *Bombus* females followed length and abdominal width measurements found in species descriptions of Mitchell (1962). Because they are crucial to local population growth throughout a season, *Bombus* queens were recorded but not retained.

Analyses of Species Characteristics

The plant preferences of each species were determined from the floral host records collected in 2012 and 2013. Because some species are rarer and some more common, sampling bias can confound comparisons between sites and between species (Gotelli 2008). Therefore, two approaches were taken to characterize and compare the diet breadth of bee species from observation data collected in standardized surveys in 2012 and 2013. In comparisons of diet breadth among bee species, rarefaction was used to standardize sample data for comparison. Rarefaction is a subsampling technique used to standardize comparisons among samples of unequal size using random draws without replacement to construct new, equal-sized datasets for each sample (Gotelli 2008). Individual-based rarefaction was used to generate a subsample of each larger sample to create a rarefied sample set with an abundance lower than the smallest sample in ECOSIM v7.0 (Gotelli and Entsminger 2001) using 1,000 iterations. Rarefication of a large sample to the size of a smaller one will inevitably result in data loss, but this can be a more profound problem in data sets with many rare (*e.g.* singleton or doubleton) observations. In the case of diet breadth analysis for these data, rarefaction to the smallest sample size overestimates the breadth of the rarest bee species by including all observations, including singletons which would be more likely to be excluded from rarefied subsets for more common species. To avoid this bias, rarefaction of plant visit data was conducted on all bee species to an abundance level of

20 per species. This is equivalent to a measure of diet breadth if plant visits of all bee species were recorded for a total of 20 visits each. Although the choice of 20 visits is somewhat arbitrary, it does allow comparisons to other studies in bee diet breadth, which have used rarefaction abundances of 10 (Goulson, *et al.* 2008; Connop, *et al.* 2010) and 20 (Williams 2005). The results of this analysis are estimates of the number of plant genera (*i.e.* richness) visited by each species that account for differences in sample size. Larger values of rarefied diet breath indicate bee species that visit a wider array of plants, and smaller values indicate those bees that visit fewer plant species.

In order to account for the large disparity in abundances among bee species and yet still retain the details of plant visit data for common species, Hurlbert's *PIE* was also calculated as an index of diversity to estimate and compare diet breadth using the full, unrarefied data in ECOSIM. The diversity of *Bombus* at each site was characterized as Hurlbert's probability of an individual encounter (*PIE*). This diversity index is robust for both large and small sample sizes and takes into account both richness and evenness (Gotelli 2008). *PIE* is calculated as

$$PIE = \left(\frac{N}{N-1}\right)\left(1 - \sum_{i=1}^{S} p_i^2\right)$$

where N=total number of plant visit observations for a bee species, S=total number of plant genera upon which a bee species was observed, i=each individual plant genus identity and p_i=the proportion of observations attributable to that plant genus. Because 14% of observations were to plants that could not be identified to species, both rarefaction and *PIE* estimates of diet breadth were calculated at the level of plant genus. The values of *PIE* range from zero to one, with minimum diversity indicated by *PIE*=0 (*e.g.* a bee species is recorded on only one genus), and

maximum diversity indicated by *PIE*=1. Interpretation of *PIE* is intuitive, with values approaching the maximum of one indicating a species that utilizes a greater diversity of food plants, and values approaching the minimum of zero indicating a more specialized species.

The relative level of specialization was estimated for each bee species following the methods of Williams (1989) and Fitzpatrick, *et al.* (2007). In brief, a contingency table was constructed with plant genera as rows and bee species as columns. The products of the marginal totals were divided by the grand total to determine the expected frequency of visits to each plant genus by each bee species assuming that all plant species were available to all bee species. These expected values are then subtracted from the observed values, and the result is divided by the expected value. The result is a relative index of the preference of each bee species for each plant genus available, with highly positive values suggesting a particular bee species' preference for a particular genus. The largest positive value is then used as a relative index to compare specialization among bee species ("relative maximum dietary preference" Fitzpatrick, *et al.* 2007). To account for variation in the availability of plants at each site (Williams 1989), only genera in the top five families present at more than half of the sites were included.

Niche overlap was estimated between pairs of bee species by calculating the Pianka index over all plant genera visits in ECOSIM v7.0 (Gotelli and Entsminger 2001). The pairwise overlap in use of plant genera between two bee species was calculated as

$$O_{12} = O_{21} = \frac{\sum_{i=1}^{S} p_{2i} p_{1i}}{\sqrt{\sum_{i=1}^{S} \left(p_{2i}^2 \right) \left(p_{1i}^2 \right)}}$$

where O_{12} and O_{21} = the symmetrical overlap between species one and species two, i = the

identity of each plant genus, S = the total number of plant genera used by both bee species, p_{1i} = proportion of each plant genus used by the first bee species in the pair and p_{2i} = proportion of that genus used by the second bee species in the pair. The index is scaled between zero, which indicates no overlap in plant use, and one, which indicates identical plant use. To test whether overlap was present among these species, significance was assessed by comparison with 1,000 simulations in which niche breadth was retained and zeroes were reshuffled for each species, and all plants were equally available to bees (recommended Randomization Algorthim 3, Gotelli and Entsminger 2001). The pairwise overlap in plant genera use was then visualized with cluster analysis with a dendrogram of dissimilarity, which was calculated as $1\text{-}O_{12}$ for each species pair, using the packages *spaa* (Zhang 2013) and *vegan* (Oksanen, *et al.* 2013) in R (R Core Team 2014).

Glossa lengths of each bee species were estimated following the protocol of Harder (1982). The length was measured as the distance between the basal sclerite of the glossa and the terminus of the flabellum using alcohol-preserved *Bombus* and *A. mellifera* workers and *X. virginica* females. Twenty two specimens for each species were measured, except for *B. fraternus*, where only 17 specimens were available.

Distribution maps within the continental United States were generated for all species included in this study from collection data available on the Global Biodiversity Information Facility (GBIF, http://data.gbif.org, accessed 12-Dec-2013), with the exception of *A. mellifera.* Because it is a managed species and usually overlooked by collectors, *A. mellifera* was assumed to be present throughout the entire continental United States. The georeferenced point data from GBIF were imported into ARCGIS v. 10.1 (ESRI, Redlands, CA), and 100 km buffers were constructed around each record. These buffers were then joined in a convex-hull, minimum

bounding polygon. This polygon was then trimmed to the boundaries of the United States to estimate the distribution of each species within the United States. In the case of *B. pensylvanicus* (DeGeer, 1773), all records in the extreme western portion of the United States were assumed to be *B. pensylvanicus sonorus* and not included in the polygon. The range maps included in Koch, *et al*. (2012) were used as a guide to determine where this cut-off would occur. In the case of *X. virginica*, southern Florida was not represented in the GBIF samples, yet other sources indicate that the species is present there (Hurd 1955; Hurd and Moure 1963). The lower portion of the peninsula was included in the distribution map created here. These distribution polygons were then used to tabulate an estimate of the area (in hectares) that each species inhabits in the continental United States with tools available in ARCGIS. All analyses were conducted with projection set at North American Datum 1983 (NAD83), Universal Transverse Mercator (UTM) Zone 15.

The activity period of each species was determined from the collection data in the standardized surveys and analyzed by week. The colony initiation date was estimated as an average of the first observation dates for each species over the three-year study. The duration of activity was determined for each species by subtracting this from the average last-observation date. The average date at which the largest numbers of each species were recorded was determined to be the peak activity date, and the duration of peak activity was calculated by adding and subtracting one standard deviation of this average.

Analyses of Site Characteristics

The diversity of bees at each site was characterized as Hurlbert's *PIE*. In this case, *N*=total number of bee observations at a site, *S*=total number of observed species at that site,

i=each individual species identity and p_i=the proportion of observations attributable to that species at that site. Again, the values of *PIE* range from zero to one, with minimum diversity indicated by *PIE*=0 (*e.g*. a single bee species at the site), and maximum diversity indicated by *PIE* = 1. *PIE* was calculated for all eight bee species, as well as just *Bombus* species alone.

Because data were not obtained on the abundances of flowers available at each site, presence-absence data was used to compare the plant communities among sites. The richness of bee-utilized plants at each site was determined by simply counting the number of plant genera visited by bees that were present at each site. For a more formal comparison, the Jaccard distance index (Gotelli and Ellison 2013) was calculated between site pairs using the *vegan* package (Oksanen, *et al.* 2013) in R (R Core Team 2014). The Jaccard index (d_{ij}) is calculated by

$$d_{ij} = \frac{a + b}{a + b + c}$$

where d_{ij}=the distance between site *i* and site *j*, *a*=the number of plant genera that occur at site *i*, but not at site *j*, *b*= the number of plant genera that occur at site *j*, but not at site *i*, and *c*=the number of genera that occur at both *i* and *j*. The Jaccard index (d_{ij}) ranges from zero to one, with d_{ij}=0 at sites that share all plant genera, and d_{ij}=1 at sites that have no plants in common. Similarities in plant communities at each site were visualized with cluster analysis using the Jaccard distance values in the *vegan* package (Oksanen, *et al.* 2013) within R (R Core Team 2014). At each site, the percentage of plants used by bees that were native, as opposed to those of exotic origin or cultivated as crops, was also calculated.

Natural color orthogonal maps for all counties used in surveys were downloaded from the United States Department of Agriculture, Natural Resources Conservation Service's Geospatial Gateway (http://datagateway.nrcs.usda.gov/). The aerial images used to generate these three-

band images were obtained through the National Agriculture Imagery Program during the growing season of 2010 and have a resolution of 1-m ground sample distance. Following the projection of these maps, all subsequent analyses were conducted with projection set at NAD83, UTM Zone 15. Sites were located on the map through importation of the latitude and longitude coordinates recorded at each site into ARCGIS v.10.1 (ESRI, Redlands, CA). The boundaries of each sampled area were determined by associating landmarks in the aerial image (*e.g.* roads, riparian areas, fence lines) with the same landmarks observed in site visits, and polygons representing the sample locations were drawn by hand. The area (in hectares) of each was measured within ARCGIS.

In order to speed processing, the county-sized orthogonal maps were trimmed to a 3 km distance surrounding each site. The pixels in these trimmed areas were then classified into land-use, land-cover (LULC) classes through supervised classification. The LULC categories were modified from standard United States Geological Service classification guidelines (Anderson, *et al.* 1967) and initially included ten classes chosen *a priori*: paved areas, bare ground, unpaved roads, buildings, grassland, farmland, hay pasture, water and forest (Table V.C.2). The ability to distinguish these classes from one another was determined by looking at scatterplots of the distribution of training pixels in each spectral band as well as trial runs of maximum likelihood classification. Preliminary analyses showed that the reflectance of water in the images overlapped with the reflectance in a number of proposed classes. To overcome this, water areas were removed from the images prior to classification and analyzed separately as a single class. For terrestrial areas, the only class that did not overlap with another was the wooded class. All other classes exhibited overlap with one or more additional classes. These were collapsed into

single, color-coded classes that best captured their overall, shared characters, particularly as they related to bee resources (summarized in Table V.C.2).

The suitability of each of these areas as bee habitat was determined *a priori* based on the likelihood of an area containing floral resources. After classification, a majority filter was applied, using four neighboring cells to remove aberrant pixels, smoothing the classes. Accuracy of the smoothed classification was assessed by comparing 160 uniformly distributed reference points to the classification map to generate a confusion matrix. This was then used to determine the overall accuracy and the kappa coefficient (Foody and Atkinson 2002). The proportion of each land cover class was determined at the sites themselves and at two radii: 250 m and 2000 m surrounding each site to analyze habitat characteristics at both local (250 m) and landscape scales (2km) (after Jha, *et al.* 2013).

To determine the influence of habitat characteristics on bee diversity and abundance, general linearized models (GLM) were employed using Poisson error distributions, with quasi-Poisson models employed to correct for under- or over-dispersion. Independent variables were checked for correlation using Pearson correlation tests, with *p*-values Bonferroni-adjusted for multiple comparisons using the package *psych* (Revelle 2014) in R (R Core Team 2014). When two variables were found to be correlated, only one was retained for use in models. Full models containing all retained independent variables were constructed and each factor was examined for significance in the model. Significant factors in the full model were retained and new models were constructed using each factor independently and in combination, but without interactions. These models were then compared against one another and a null model using χ^2 tests, with alpha levels Bonferroni-adjusted for multiple comparisons.

D. RESULTS

Between May 16, 2011 and October 20, 2013, 412 standardized surveys of 13 sites in Northwest Arkansas were conducted. Throughout the study, 1,693 *Bombus*, 1,058 *X. virginica* and 3,191 *A. mellifera* were observed at the 13 study sites during a total of 200 sampling hours. Pearson correlations between total observations of each species and the rate of bees hr^{-1} ranged from 98–100%, suggesting that using either rates or raw abundances of species were equivalent in analyses. In descending order, the relative abundances of each species were: *A. mellifera* (53.7%), *X. virginica* (17.8%), *B. griseocollis* (De Geer, 1773) (14.74%), *B. impatiens* (6.9%), *B. pensylvanicus* (2.9%), *B. bimaculatus* Cresson, 1863 (2.2%), *B. auricomus* (Robertson, 1903) (1.1%) and *B. fraternus* (Smith, 1854) (0.66%).

A total of 4,007 bees were recorded foraging for nectar and pollen in 2012–2013. Most plants visited by bees were identifiable to species (n=3,451), although 14% (n=556) of observations were to plants that were not discernable below the level of genus, and 0.4% (n=15) were only identifiable to family (Asteraceae, a thistle that could have been either *Carduus* or *Cirsium*). Complete lists of floral records for each species are given in Appendix V.H.1. Bees visited 32 families of plants, 88 plant genera and 102 identified plant species (127 morphotypes total). Of these 102 plant species, 68% (n=69) were native, 24% (n=24) were exotic and 9% (n=9) were crops.

The eight bee species had marked differences in their use of native versus exotic (including crop) species of plants (Test of Independence, χ^2 =507.9, df=7, p<0.001), with most species more commonly observed on native plant species (Table V.D.1). Over 90% of observations of *B. auricomus, B. fraternus* and *B. impatiens* were on native plant species. *Bombus griseocollis, B. pensylvanicus* and *X. virginica* were observed on native plants >75% of

the time. *Apis mellifera* was more common on crop plants than the other species (14.9% of observations), but nearly half of the observations of this species were on native plants (48.4%). *Bombus bimaculatus* was the only species that was more commonly observed foraging on exotic plant species (72.1% of observations). The bulk of *B. bimaculatus* foraging observations (63.8%) were on the exotic vetch species *Vicia villosa* (winter vetch) and *V. sativa* (garden vetch). The top eight plant species for each bee species are presented in Appendix V.H.2.

Diet breadth was estimated for the eight targeted bee species in these surveys using genera-level plant visitations (Table V.D.2). The average rarefied diet breadth over all species ranged from 6.58 (*B. bimaculatus*) to 12.3 (*A. mellifera*) plant genera with an average of 10.1±2.08 SD genera. The average *PIE* diet breadth ranged from 0.59 (*B. bimaculatus*) to 0.93 (*B. pensylvanicus*) with an average of 0.85±0.12 SD. Five families (Asteraceae, Lamiaceae, Fabaceae, Passifloraceae and Rosaceae) were most commonly available, based on their occurrence on greater than half of the sites surveyed (n sites=13, 12, 12, 10, 7, respectively). Collectively, these families accounted for 76% of bee visits (n=3,046), ranging from 65– 92% of the total observations for each bee species. The 48 genera within these families were used to compare relative levels of specialization among species by calculating the maximum dietary preference exhibited by each species in this subset of commonly available plant genera. Levels of specialization (relative maximum dietary preference) ranged from 0.86 (*A. mellifera,* a six-way tie among genera) to 27.20 (*B. auricomus, Baptisia*). Values of the maximum dietary preference for each species are given in Table V.D.3.

Analysis of niche overlap among species pairs showed significant overlap ($p<0.001$, 1,000 simulations), rejecting the null hypothesis of no overlap in resource utilization among these species. Pairwise estimates of overlap (estimated as the Pianka index O_{12}) ranged from 0.11

(*B. bimaculatus* and *B. fraternus*) to 0.64 (*B. fraternus* and *B. impatiens*) (Table V.D.4). Summarizing all pairwise Pianka distances (1- O_{12}) through cluster analysis shows three main clusters: *A. mellifera* + (*B. fraternus* + *B. impatiens*), *B. auricomus* + *B. pensylvanicus* and *B. griseocollis* + (*B. bimaculatus* + *X. virginica*) (Fig. V.D.1). Although *B. fraternus* and *X. virginica* had a high estimate of niche overlap (O_{12} = 0.41), these species were quite distant in the cluster analysis, which incorporates all pairwise relationships in determining clusters.

Average glossa lengths ranged from 3.59 mm (*A. mellifera*) to 6.35 mm (*B. pensylvanicus*) (Table V.D.3). The glossa lengths of the species *B. auricomus* and *B. pensylvanicus* were longer (means with non-overlapping standard deviations) than those of all species but *B. bimaculatus.* The glossa length of *A. mellifera* was much shorter than that of *B. bimaculatus, B. auricomus* and *B. pensylvanicus.* A strong linear relationship (R^2 = 0.77, p = 0.004) was observed between glossa length and maximum dietary preference, indicating that long-glossa species were more likely to exhibit relatively strong dietary preferences than shorter-glossa species (Fig. V.D.2). Additionally, the amount of resource-use overlap among species pairs as estimated by the Pianka index was negatively correlated with the absolute value of the difference in glossa lengths in each pair (Pearson's, r_{27} = -0.45, p = 0.02, Bonferroni-adjusted).

Range sizes of each species in this study were estimated from point data, and ranged from 322.2 million ha (*B. auricomus*) to 855.3 million ha (*A. mellifera,* for this species the range was estimated as the entire continental United States) (Table V.D.3). *Bombus griseocollis* was the only bumble bee species present in Arkansas that had a range extending to the western coast of the United States. Range maps for all species are provided in Appendix V.H.3. These distribution maps clearly show that Northwest Arkansas is firmly within the ranges of all seven native bee species. A comparison of the range size and the number of records of each species in GBIF

allowed a generalized assessment of how common each species is throughout its range. *Bombus fraternus* was the least common, with a density of 1.5 GBIF records per 10^6 ha, and *B. impatiens* was most common, with a density of 33.2 records per 10^6 ha (Table V.D.3). The community composition differed quite a bit in the Northwest Arkansas region, using these records as a proxy for range-wide commonness. The three species with the highest relative abundance in the GBIF density measures were *B. impatiens* (34.6%), *B. pensylvanicus* (21.3%) and *A. mellifera* (13.6%), while the top three species in Northwest Arkansas were *A. mellifera, X. virginica* and *B. griseocollis*, as previously listed. *Xylocopa virginica* was relatively uncommon throughout its range, with density of 2.5 records per 10^6 ha and a relative abundance of 2.6%, as compared to its relative abundance in Northwest Arkansas of 17.8%. *Bombus fraternus* was relatively less abundant than all other species, both throughout its range (1.6%) and the study region (0.66%).

Bees were active from week 14 (mid-March) until week 43 (late October), and species exhibited different seasonal activity periods (Fig. V.D.3). *Apis mellifera* was typically the first species observed (average start week=16.67, early April) and exhibited the longest adult activity period (25.67±3.21 SD weeks). *Xylocopa virginica* was similar, with an average start week of 17.33 (early-April) and an active duration of 23.67±1.53 SD weeks. Among the bumble bees, *B. griseocollis, B. auricomus* and *B. bimaculatus* were the earliest to start activity (average start weeks, 18, 18.33 and 18.33, mid-April). *Bombus griseocollis* had a relatively long active period of 19±3 SD weeks, but *B. auricomus* and *B. bimaculatus* had the shortest periods of activity (11.33±3.51 SD and 10±2.65 SD weeks, respectively). *Bombus pensylvanicus* had the latest start week (26, early June), followed by *B. fraternus* (average start week=25.33, late-May). *Bombus impatiens* had the longest active period of any of the bumble bees, with an average active duration of 22.3±4.62 SD weeks. All species were sighted unusually early in 2012, but the

earliest sighting of *B. fraternus* was 11 weeks prior to the average initiation of activity, far out of season compared to other species (Fig. V.D.3). The raw abundances of each species by week and by cumulative degree days are shown for all three years in Appendix V.H.4.

The accumulation of degree days was inconsistent over the three-year period, and this might have influenced the dates at which bee activity began each year. Figure V.D.4 shows degree-day accumulation by year. From these data we can see that 2012 was warmer than the other sample years, and that 2013 was cooler. For example by week 14 of 2012, the cumulative degree days estimate was 486.4±8.0 SE, but 2011 would not accumulate that amount until week 17, and it would take until week 19 for 2013 to match that accumulation. Precipitation data showed that 2012 was also much drier, with an average total accumulation of 25.8±0.002 SE inches by week 43, as compared to 44.1±0.09 SE inches in 2011 and 48.6±0.32 SE inches in 2013 (data not shown). The earliest week in which each species was spotted also varied yearly, with all species spotted earlier in 2012 than in 2011 or 2013 (Fig. V.D.5). A comparison of activity by week and by cumulative degree days can be visually conducted with graphs for each species in Appendix V.H.4.

The bee communities differed widely among the 13 sites surveyed (Fig. V.D.6). Total bee abundance at each site ranged from 124 to 969, with an average 457±265 SD bees observed at each site, totaled over the three year study period. *Bombus* abundance at each site ranged from 15 to 506, with an average of 130±127 SD bumble bees per site. *Apis mellifera* and *X. virginica* were present at all 13 sites and accounted for 54% and 18% of all bee observations, respectively. *Apis mellifera* accounted for 27–90% of bee observations at each site and was the most common species observed at all but two sites (Chesney and LakeFay). At both Chesney and LakeFay, *X. virginica* was the most common species (39% and 46%, respectively), although *X. virginica* was

less common at other sites (3– 30%). Baker, Searles and Woolsey were the only sites at which all eight species were observed. *Bombus griseocollis* was the most commonly observed bumble bee species at most sites (2–31%), and it was the only species of bumble bee observed at the Madison site. Across all 13 sites, *B. griseocollis* (n=876) accounted for 52% of all *Bombus* observations and 15% of all bee observations. *Bombus impatiens* was the most common bumble bee at the Baker and Golden sites (22% and 16%, respectively), and *B. pensylvanicus* was the most common at the World site (8%). *Bombus fraternus* was only present at seven sites, and *B. auricomus* was only present at eight sites (Fig. V.D.6). Total bee diversity ranged from 0.18–0.78, with a mean *PIE* of 0.59±0.18 SD across sites. Bumble bee diversity ranged from 0–0.73, with a mean *PIE* of 0.53±0.20 SD.

Plant richness, as measured by the number of bee-visited genera present at each site, ranged from 11 (Madison) to 28 (Baker), with an average of 18.2 ± 0.26 SD (Table V.D.5). Lots had a uniformly low plant richness, ranging from 11 (Madison) to 16 (Sunrise) genera. The percentage of plant species that were of native origin at each site ranged from 26–97%, with an average of 66%±26% SD. Prairie sites were uniformly dominated by native plant species, with 97% (Baker) to 90% (Chesney) of bees observed on native plant species. The two farms exhibited low native plant composition, with only 26% of bee observations on native plants at Horn and 39% at Dickey. A comparison of the plant community composition among sites was conducted by calculating pairwise Jaccard distances between site pairs, the results of which are shown in Table V.D.5. The prairie sites Searles and Chesney were most similar, with a Jaccard distance of 0.55. The most dissimilar site pair was Madison and Searles, with a Jaccard distance of 0.97. Cluster analysis showed five plant community clusters 1) the Lot sites Madison +

Carroll, 2) the Farm sites Horn and Dickey, 3) Golden + (Sunrise + Noland), 4) Baker, 5) (Woolsey + (Searles + Chesney)) + (Lake Fay + World) (Fig. V.D.7).

The thirteen sites varied in the proportion of land cover in each of four classes: developed, wooded, herbaceous and water. The overall classification accuracy was 98.1%, with a kappa coefficient of 97.5%. Figure V.D.8 shows the area surrounding all13 sites within the greater landscape area (left), and an example (Woolsey) of the classification results. Only two sites had water present, and both of these in low amounts (<2% cover). Water was discarded as a potential factor prior to analyses. The percentage of herbaceous land at 250 m was negatively correlated with the percentage of that radius that was wooded (r=-0.85, p=0.04). Because of this correlation, only one of these cover classes was retained in modeling. In terms of bee habitat, wooded areas are more homogenous and less likely to include floral resources throughout most of the year. Because the herbaceous cover class could not distinguish between bee-friendly habitat rich with flowers and pastures and other grasslands with little floral resources, wooded land was retained for analysis. Wooded cover ranged from 0–65% at each site, with an average of 16%±20% SD. The percentage of developed land at each site ranged from 0–58%, with an average of 17%±19% SD (Table V.D.6).

GLM analyses were conducted at each scale (site, local and landscape) independently, with bee abundance, bee diversity, *Bombus* abundance, *Bombus* diversity and abundance of each species individually as dependent variables (Appendix V.H.5). Factors with p-values less than the Bonferroni-adjusted significance thresholds were retained within each model. Overall bee abundance increased with increasing plant richness at each site ($F_{(1,11)}$=45.62, p<0.001; Fig. V.D.9). To test the accuracy of this relationship, a GLM was tested independently using the abundance data from 2011, the year in which plant host data was not quantified. The same model

was recovered, with total bee abundance at a site best predicted by the number of plant genera alone ($F_{(1,11)}$=10.7, p<0.01). This relationship was also reflected in the abundance of all species of bumble bees as a group and individually for four *Bombus* species (Table V.D.7), although *B. bimaculatus* and *B. griseocollis* abundances were independent of all site-level factors analyzed here. Differences in *A. mellifera* and *X. virginica* abundances among sites were not explained by any site-level factors. Differences in *B. fraternus* abundance were best explained by a more complex model (Table V.D.7). The abundance of *B. fraternus* was positively associated with plant richness and the area of the site and negatively associated with the percentage of developed land at each site. *Bombus fraternus* was the least frequently encountered bee in the study (0.7% of all bee observations, 2.3% of all *Bombus* observations) and was only present at seven of the thirteen sites (Fig. V.D.6). No site-based models explained differences in total-bee diversity or *Bombus*-specific diversity among sites.

As in the case of site-level analyses, wooded cover was retained over herbaceous cover as a factor in the local- and landscape-level models. At the local scale, wooded cover ranged from 4–64%, with an average of 30%±17% SD (Table V.D.6). The percentage of developed land ranged from 5–43%, with an average of 22%±11% SD at a radius of 250 m surrounding each site. Land cover classes did not explain differences in bee diversity or in abundances among sites at a local scale. At the landscape scale, wooded cover ranged from 16– 47%, with an average of 31%±10% SD. The percentage of developed land ranged from 7–40%, with an average of 21%±12% SD at a radius of 2000 m surrounding each site. The proportion of land cover at the landscape scale did not explain differences in diversity or the abundance of most bee species. The abundance of *X. virginica* was negatively associated with the proportion of wooded cover in a 2000 m radius surrounding each site ($F_{(1,11)}$=10.77, p<0.01, Fig. V.D.10).

The abundance of honey bees, carpenter bees and some bumble bee species was positively associated with the richness of plants at these sites surveyed in Northwest Arkansas. Additionally, *B. fraternus* abundance was positively associated with the area of the site and negatively associated with the amount of development at the site itself. Site characteristics such as plant richness, percent native species and land cover were not associated with measures of overall diversity among these species or bumble bee diversity alone. Neither diversity nor abundance was associated with any measures of land cover at a local level (250 m). At the larger landscape level (2000 m), the abundance of *X. virginica* was negatively associated with the percent of landscape covered in forest. No other measures of abundance or diversity were associated with landscape-level land cover.

E. DISCUSSION

Northwest Arkansas Bee Community

One broad goal of this project was to gain knowledge of a bee community in Northwest Arkansas in order to provide an ecological foundation that could be of use in regional bee conservation. The eight species under consideration here are likely an important community of pollinators in the region. This community is active throughout the frost-free growing season in the region and collectively visited 32 families of plants, 88 plant genera and 102 identified plant species, most of which were native. Three species, *A. mellifera, X. virginica* and *B. griseocollis,* were much more abundant than the other five species and collectively accounted for 86% of observations. The non-native *Apis mellifera* is an important member of the bee community in Northwest Arkansas. It was the most abundant species encountered at these sites, with a 26-week active period that overlapped with all seven of the native species under consideration here (Fig. V.D.3). *Apis mellifera* showed a wide diet breadth (Table V.D.2) with little discrimination

among the plants commonly available in the area (Table V.D.3) and was observed on 91 species of plants in 69 genera (Appendix V.H.1). It showed the greatest niche overlap with *B. fraternus* and *B. impatiens* (Fig.V.D.1), with whom it also overlapped in glossa length (Table V.D.3). The second-most abundant species encountered at these sites was *X. virginica.* Unlike elsewhere in its range, *X. virginica* is abundant in Northwest Arkansas, and likely an important species within this bee community. Like *A. mellifera*, *X. virginica* had a long active season (24 weeks) that nearly spanned the entire season in the region (Fig. V.D.3). Also similarly, it had a wide diet breadth (Table V.D.2) and showed little specialization (Table V.D.3), with observations on 68 plant species within 48 genera (Appendix V.H.1). *Xylocopa virginica* shared the floral niches of *B. bimaculatus* and *B. griseocollis* (Fig. V.D.1) and showed a large overlap with *B. fraternus* as well (Table V.D.4), all of which overlapped in glossa length (Table V.D.3). Although I did not measure competition explicitly, these characteristics suggest the potential for competition among *A. mellifera*, *X. virginica* and *Bombus* in the region.

Resource Overlap and Phenological Separation

Although the bees studied here are generalists, their use of plant resources is not uniform. There is degree of niche partitioning in this bee community that is ascribable to both the length of their glossae and their seasonal phenology. The differences in glossa lengths among bumble bee species intuitively correspond to the species' use of floral resources. On a per flower basis, long-glossa species are more efficient at gathering nectar from long-throated flowers and short-glossa species are similarly aligned with short-throated flowers (Hobbs, *et al.* 1961; Inouye 1980; Heinrich 2004). There is much evidence in favor of interspecific competition for nectar among bee species within a community. First, there is a high potential for nectar to be limiting within a habitat. When the quantity of nectar available was compared to that removed by bees

among plants preferred by bumble bees in Maine, 92% of this resource was removed on a daily basis on average over the season (Heinrich 1976). This limitation of nectar suggests that nectar acquisition might be competitive among the members of a bee community. Secondly, *Bombus* can detect the mere "footprint" of other bees and will actively avoid flowers that have been visited (Goulson, *et al.* 1998). This behavior is learned through experience (Leadbeater and Chittka 2011), and the capacity to learn to detect previously depleted resources suggests that encountering flowers drained of nectar by other bees could be a common experience. Also, selective exclusion experiments show that bumble bees expand their resource base, utilizing flowers of the "wrong" corolla size when other *Bombus* species are excluded (Inouye 1978). Lastly, experimental addition of *A. mellifera* to isolated natural sites in which they were absent showed a highly negative effect on native *B. occidentalis* Greene, 1858, which had smaller gyne-to-worker outputs in the presence of high densities of *A. mellifera* (Thomson 2004).

Based on the differential nectar foraging efficiency driven by glossa lengths in Rocky Mountain *Bombus* communities, Inouye (1978) hypothesized that a strict competitive-exclusion principle applies to bumble bees: a resource-limited site can only support, at most, four species of bumble bees: one each of a long-, short- and medium glossa and one nectar robber that obtains nectar by piercing the corolla. This hypothesis was further confirmed with the work of Pyke (1982) in the same region of the Rocky Mountains. The isolation of floral patches within this study region presented an ideal scenario in which each bumble bee community was isolated in a resource-limited patch, thus rendering the effects of competition more visible. Deviations from this hypothesized four-member bumble bee community are thought to be the result of overlapping populations in areas of non-limiting resources (Pyke 1982). The bee communities I studied in Northwest Arkansas support more species than this hypothesis allows. While these

populations are unlikely to be as isolated as those in mountain meadows and might not be resource limited, phenological separation might offer additional insights into this disparity between theory and observation. Also, *A. mellifera* and *X. virginica* are members of this community as evidenced by their spatial, temporal and resource overlap with the resident bumble bees. How do these bees fit within the hypothesized community?

The early-season bee community in Northwest Arkansas begins with the appearance of the bumble bees *B. auricomus, B. bimaculatus* and *B. griseocollis* along with *A. mellifera* and *X. virginica.* The early-season bumble bees fit Inouye's (1978) prediction quite well, with one each of a short (*B. griseocollis*), medium (*B. bimaculatus*) and long (*B. auricomus*) glossa species. *Bombus impatiens* joins the community soon enough to overlap with *B. bimaculatus* and *B. auricomus,* although it does not begin to peak until after *B. auricomus* and *B. bimaculatus* have completed their relatively short colony cycles (Fig. V.D.3). *Bombus impatiens* is a good candidate for the open slot Inouye (1978) reserved for a nectar robber in his resource-limited bumble bee communities. Although I did not quantify nectar-robbing behavior, *B. impatiens* was the bumble bee species I most commonly observed robbing nectar. Whether or not *B. impatiens* created their own access holes (were primary robbers) or merely took advantage of those created by *X. virginica* (were secondary robbers) is unknown. *Xylocopa virginica* was frequently observed robbing nectar during this study, and it has a solid reputation for nectar larceny. The galea morphology of *Xylocopa* is unique among the bees, with interlocking edges that allow carpenter bees to force their mouthparts through the flesh of flowers to rob the nectar contained within (Krenn, *et al.* 2005).

The tidy bumble bee community suggested by Inouye's (1978) work in the short season of subalpine Colorado begins to break down as the season progresses in Northwest Arkansas.

Although the extended growing season allows for more complicated interactions, phenological differences seem to account for some, but not all of the disparity between Inouye's hypothesis and observations in Northwest Arkansas. The two long-glossa species that are present at these sites are *B. auricomus* and *B. pensylvanicus.* Although these two species show strong overlap in their plant uses (Fig. V.D.1, Table V.D.4) and high degrees of specialization (Table V.D.3), they are greatly separated by phenology and show little temporal overlap (Fig. V.D.3).This suggests that these two species, which could be heavy competitors for similar floral resources, might parse themselves out according to phenology to avoid competition at these sites. The temporal overlap between *B. pensylvanicus* and *B. auricomus* is minimal, and the short peak period of *B. pensylvanicus* does not occur until after *B. auricomus* has completed its cycle. The short glossa *B. griseocollis* and *B. impatiens* are already present when the late season, short glossa *B. fraternus* and long glossa *B. pensylvanicus* arrive on the scene. The overlap in both plant use and phenology between *B. fraternus* and *B. impatiens* is more puzzling. The abundance of these two species among sites was positively correlated (Pearson's, r_6=0.85, p=0.005, Bonferroni-adjusted) as well, which suggests that they comfortably use the same sites, resources and season without inducing competitive exclusion. Throughout its range, *B. fraternus* is rather uncommon while *B. impatiens* tends to be quite common (Table V.D.3). Perhaps the overlap between these species keeps *B. fraternus* uncommon, yet it is capable of sustaining itself in areas without resource limitation.

The species with the greatest overlap in glossa length are *B. impatiens, B. griseocollis, B. fraternus* and *X. virginica* (Table V.D.3). Although they also show overlap in their overall phenology (Fig. V.D.3), their relative abundances change over the season (Fig. V.D.11). *Xylocopa virginica* has a bimodal abundance throughout the year, with both early and late peaks

and a mid-season lull of low abundance (Fig. V.D.11). This mid-season drop in both the relative abundance and raw abundance of *X. virginica* is reflected in the abundances of both sexes, and could reflect the unusual life history of *X. virginica.* Unlike other bees, *X. virginica* overwinter as adults, but do not reach sexual maturity until the following year (Gerling and Hermann 1978). Mating takes place in spring (confirmed locally with a single personal observation on April 30, 2013), and new adults appear in late summer (Balduf 1962; Gerling and Hermann 1978). There is an obvious reduction in numbers of adult *X. virginica* in the interim, and it is during this period that *B. griseocollis* populations peak and *B. impatiens* numbers begin to grow (Fig. V.D.11, Appendix V.H.4). Thus, although these species overlap in glossa length and general phenology, a finer scale analysis of their abundances over time suggest that phenological separation either mitigates direct competition or that competition drives relative phenological patterns in at least parts of this bee community.

Like other animals and plants, the development of bees and their floral resources are largely dependent upon temperature. Temperature seems to be the key factor for queen emergence and nest initiation (Alford 1969). Year to year, calendar dates might not accurately reflect the passage of seasons in biological terms. Although factors other than temperature are certain to play a role in bee phenology, a comparison of abundances by week and by degree day across years gives some support for a strong role of temperature in governing bee phenology in this region (Appendix V.H.4). For early-season species in particular, peaks of activity align more readily across years when abundance is compared to cumulative degree days than when compared by calendar week (*e.g. B. auricomus, B. bimaculatus, A. mellifera* and *X. virginica*, Appendix V.H.4). This effect is lessened in the later emerging species *B. pensylvanicus.*

Community Composition among Sites

A uniform distribution of species would suggest that all eight species targeted in this study should be present at all 13 sites, yet this is not the case. *Bombus fraternus* is absent from six sites and *B. auricomus* is absent from five sites (Fig. V.D.6). Why does the species composition vary between sites that are so close geographically? Although these species are somewhat rarer when they are present (relative abundance: ~1% each), *B. bimaculatus* and *B. pensylvanicus* are comparably rare in terms of abundance, yet both are present at more sites (relative abundance: 2.2% and 2.9%; 11 and 12 sites, respectively). Only the three most common species, *B. griseocollis, A. mellifera* and *X. virginica*, were present at all 13 sites. Also, there was no correlation between the diversity and abundance of bees at these sites (Pearson's, r_{11}=-0.13, p=0.66, Bonferroni-adjusted), suggesting that diversity and abundance are driven by separate factors. Are there site characteristics that dictate these differences in community composition? The diversity of plants at each site was a strong predictor of abundance (Fig. V.D.9), but no site or landscape variables explained the diversity of bees at these sites. The lack of an effect of the surrounding habitat on the diversity or abundance of most species is surprising. The absence of *B. fraternus* from eight sites is likely driven by the association between this species and site-based variables. The higher abundance of *B. fraternus* at sites that were large, rich in plant genera and had lower amounts of development (GLM, Table V.D.7) suggests that this species has some habitat requirements that were not always met at the sites in this study. Other studies have compared the differences in *Bombus* diversity among sites and found that the abundance of floral resources can affect the diversity of species present (*e.g.* Williams 1989). If resources are limiting at particular sites, species might be eliminated through competitive exclusion. I did not measure the density or abundance of floral resources in the course of this study, but perhaps

these factors could better explain the differences in species composition among sites. The Madison site was particularly vulnerable to late-summer drought conditions, with no bees and few flowering plants observed between late June and late August, with the exception of 2013, which was the coolest (Fig. V.D.4) and wettest summer during the study (data not shown). This resource depauperate site also had the fewest bees present, with only the three most common species spotted there: *B. griseocollis, X. virginica* and *A. mellifera* (Fig. V.D.6).

Summary and Application to Regional Conservation Efforts

In this study, I tested the following hypotheses 1) some bee species will overlap in their use of floral resources, while others will occupy more specialized niches, 2) *Bombus* species are temporally specialized, with divergent phenologies and 3) site-based characteristics such as land use and floral diversity will help explain both the differential abundance and diversity of bees among sites and that different species will respond to these factors at different spatial scales. I found some support for parts, but not all of these hypotheses. Regarding floral niches, the long glossa species *B. auricomus* and *B. pensylvanicus* are more specialized and show high niche overlap with one another. The phenological data I present here support the notion that their divergent phenologies prevent direct competition between these two species for floral resources. This is a somewhat novel finding that might help explain why bumble bee communities are more speciose than expected under Inouye's (1978) hypothesis of strict competitive exclusion by glossa length. I did not find support for the hypothesis that site or landscape characteristics could explain differences in diversity among sites, but that most species are more abundant when the site itself has a wide diversity of flowering plants.

This last finding is perhaps the most useful to local land managers looking to increase their populations of bees. A wide diversity of flowering plants used by bees locally is likely to increase population numbers on the whole. Particular species have been shown to favor particular plant genera, (*e.g. B. auricomus* and *B. pensylvanicus* both favor wild indigoes (*Baptisia*)*; B. auricomus* additionally favors beebalms (*Monarda*)*; B. pensylvanicus* favors ironweeds (*Vernonia*) and *B. fraternus* favors beggarticks (*Bidens*)). These plants could be planted to help enhance the available resources for these particular species. Combined with knowledge of the seasonal phenology and diet breadth of each of these bees, farmers should be able to increase the abundances of pollinators appropriate for their crop blooming needs.

F. Acknowledgements

I would like to thank David Dickey and Faye Horn for access to their lovely farms, Sid and Chad at the ranch for access to their fascinating site, Joe Woolbright for his excellent restoration and maintenance of prairies throughout Arkansas and information on the history of Chesney Prairie, Bruce Shackleford for the impressive restoration of Woolsey Wet Prairie and his dedication to regional prairie restoration, Aubrey Shepard for his enthusiasm for keeping World Peace Prairie wild, Michelle Strange, Duyen Tran and Bruce Richart at CH2M Hill for access to Paul R. Noland and their commitment to creating wildlife habitat, Bob Caulk, Terri Lane and Judy Woltjen with the Fayetteville Natural Heritage Association for access to restoration sites, background information and their unrelenting dedication to protecting natural areas in in the city of Fayetteville, Raghu Sathyamurthy for assistance in R programming and the Arkansas Natural Heritage Commission for access to prairie sites under permit numbers S-NHCC-12-013 and S-NHCC-13-010.

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Table V.C.1. Site information summary.

Site	Type	Elevation (m)	Area (ha)	Latitude	Longitude
Baker	Prairie	357.5	11.45	36.244444	-93.132778
Carroll	Lot	458.4	1.26	36.137074	-93.530634
Chesney	Prairie	362.1	24.31	36.221292	-94.484128
Dickey	Farm	351.4	4.32	36.16454	-94.307181
Golden	Lot	363.0	1.17	36.132194	-94.147811
Horn	Farm	342.9	4.15	36.205747	-94.256925
Lake Fay	Habitat	389.2	8.62	36.147114	-94.124314
Madison	Lot	440.4	0.72	36.131585	-93.852362
Noland	Habitat	355.1	2.94	36.082631	-94.088775
Searles	Prairie	397.2	3.01	36.356395	-94.144186
Sunrise	Lot	385.0	3.69	36.022347	-94.174228
Woolsey	Habitat	374.0	19.27	36.067094	-94.233578
World	Habitat	375.8	0.84	36.051894	-94.172728

Table V.C.2. Land-use / land-cover (LULC) classification system employed in this study

LULC Category	Description	Color	Bee Habitat
Developed	Areas that contain buildings, paved roads, unpaved roads and bare ground.	Red	No
Wooded	Forested areas, including riparian zones.	Dark Green	No
Herbaceous	Areas of herbaceous cover, including prairies, pastures and farms.	Green	Yes
Water	Areas with water visible in aerial imagery, including ponds and creeks.	Blue	No

Table V.D.1 Percentage of observations of each species made on crop, exotic or native plants in 2012–2013.

Bee species	Crop	Exotic	Native
B. auricomus	0.0%	9.8%	90.2%
B. bimaculatus	0.0%	72.1%	27.9%
B. fraternus	0.0%	2.7%	97.3%
B. griseocollis	0.8%	23.7%	75.5%
B. impatiens	3.9%	5.2%	90.9%
B. pensylvanicus	9.3%	9.3%	81.4%
X. virginica	1.5%	17.5%	80.9%
A. mellifera	14.9%	36.7%	48.4%

A χ^2 test of independence indicated that use of native versus exotic + crop plant species significantly varied among bee species, χ^2=507.9, df=7, p<0.001.

Table V.D.2 Diet breadth among bees surveyed during standardized sampling bouts in Northwest Arkansas in 2012–2013.

Bee species	N	No. plant species[a]	No. plant genera	Rarified diet breadth	*PIE* diet breadth
B. auricomus	51	13	12	7.53	0.77
B. bimaculatus	74	15	13	6.58	0.59
B. fraternus	37	18	16	11.21	0.91
B. griseocollis	530	49	37	10.95	0.92
B. impatiens	332	46	33	9.21	0.85
B. pensylvanicus	118	28	24	11.48	0.93
X. virginica	672	68	48	11.44	0.91
A. mellifera	2183	91	69	12.31	0.92
Totals	3992	127	88	14.16[b]	0.96[b]

N=number of bee observations, No.=number of observations. Diet breadth estimated at the genera level, with rarefaction to a subset abundance of 20 visits per bee species and *PIE* calculated over the complete set of observations. [a] =species or morphotypes, [b] =as calculated for the bee dataset as a whole.

Table V.D.3. The species-specific characteristics, maximum dietary preference, glossa length, range size within the United States and number of GBIF records, estimated in this study.

Species	Maximum Preference	Glossa Length (mm ± SD)	US Range Size (ha x 10^6)	No. GBIF Records
B. auricomus	27.2	6.29 ± 0.57	322.2	1773
B. bimaculatus	7.19	5.19 ± 0.69	355.2	4045
B. fraternus	13.43	4.56 ± 0.79	337.9	512
B. griseocollis	7.11	4.53 ± 0.73	629.6	5278
B. impatiens	8.94	4.51 ± 0.71	388.2	12886
B. pensylvanicus	18.23	6.35 ± 0.98	554.3	11326
X. virginica	4.82	4.43 ± 0.95	340.2	860
A. mellifera	0.86	3.59 ± 0.50	855.3	11188

Table V.D.4. Pairwise estimates of overlap in plant use between all bee species pairs.

	B. auri	*B. bima*	*B. frat*	*B. grise*	*B. impa*	*B. pens*	*X. virg*
B. auricomus	-	-	-	-	-	-	-
B. bimaculatus	0.182	-	-	-	-	-	-
B. fraternus	0.042	0.011	-	-	-	-	-
B. griseocollis	0.266	0.310	0.327	-	-	-	-
B. impatiens	0.037	0.032	**0.640**	0.140	-	-	-
B. pensylvanicus	**0.540**	0.214	0.189	0.297	0.135	-	-
X. virginica	0.172	**0.462**	**0.405**	0.315	0.141	0.236	-
A. mellifera	0.115	0.202	0.285	0.301	0.236	0.181	0.280

Estimated via the Pianka index. Overlap was greater than expected when compared with 1,000 simulations, $p<0.001$. Species pairs with overlap greater than the mean plus one standard deviation (0.239 + 0.148) are noted in bold. Species names are abbreviated to the first four letters of the specific epithet in the column headers.

Table V.D.5. A pairwise comparison of the diversity of plant genera found at each site.

Sites (Type)	Dickey (23)	Horn (20)	Searles (23)	Chesney (19)	Baker (28)	Madison (11)	Carroll (13)	Sunrise (16)	Golden (15)	LakeFay (14)	Woolsey (19)	World (15)	Noland (20)
Dickey (F)	-	-	-	-	-	-	-	-	-	-	-	-	-
Horn (F)	0.567	-	-	-	-	-	-	-	-	-	-	-	-
Searles (P)	0.930	0.925	-	-	-	-	-	-	-	-	-	-	-
Chesney (P)	0.895	0.946	0.552	-	-	-	-	-	-	-	-	-	-
Baker (P)	0.937	0.909	0.692	0.730	-	-	-	-	-	-	-	-	-
Madison (L)	0.937	0.931	0.970	0.929	0.946	-	-	-	-	-	-	-	-
Carroll (L)	0.909	0.969	0.941	0.897	0.892	0.800	-	-	-	-	-	-	-
Sunrise (L)	0.818	0.875	0.853	0.793	0.900	0.773	0.739	-	-	-	-	-	-
Golden (L)	0.774	0.833	0.914	0.903	0.951	0.960	0.880	0.760	-	-	-	-	-
LakeFay (H)	0.879	0.937	0.806	0.731	0.895	0.958	0.773	0.696	0.840	-	-	-	-
Woolsey (H)	0.833	0.917	0.727	0.690	0.854	0.929	0.933	0.833	0.903	0.731	-	-	-
World (H)	0.914	0.833	0.774	0.786	0.806	0.917	0.880	0.760	0.889	0.739	0.828	-	-
Noland (H)	0.771	0.788	0.868	0.818	0.884	0.852	0.778	0.560	0.750	0.828	0.853	0.833	-

The number in parentheses after each site indicates the numbers of plant genera bees were observed visiting at each, the letters indicate site types: F=farm, P=prairie, L=lots and H=habitats. The diagonals indicate the Jaccard distance index. Sites that share no plants will have an index of 0, while those that have identical community composition will have an index value of 1.

Table V.D.6. Measures of independent variables included in GLM models for each site.

		Site-Level			Local-Level		Landscape-Level	
Site	Richness	% Native	% Develop	% Wooded	% Develop	% Wooded	% Develop	% Wooded
Baker	28	96.8	0.6	23.6	27.9	20.3	30.3	26.3
Carroll	13	64.3	41.4	0.0	24.7	32.7	12.0	42.5
Chesney	19	90.0	0.0	15.8	4.5	6.2	12.3	15.6
Dickey	23	39.1	40.9	2.3	13.2	39.6	6.8	46.8
Golden	15	37.5	11.6	10.8	37.2	30.8	38.8	25.6
Horn	20	26.3	57.6	0.6	12.6	39.6	11.0	34.6
LakeFay	14	88.2	26.6	40.9	15.6	64.0	36.8	23.2
Madison	11	41.7	14.3	0.0	14.2	30.0	9.4	32.8
Noland	20	50.0	23.5	11.6	43.0	30.2	14.0	45.7
Searles	23	96.0	0.0	3.6	25.3	5.2	20.4	19.3
Sunrise	16	55.6	1.9	37.0	16.5	47.2	20.7	40.3
Woolsey	19	94.7	2.1	1.0	20.0	3.9	20.6	22.0
World	15	80.0	0.5	65.2	31.4	37.5	40.0	33.9

Richness and % Native refer to measures of plant genera at each site. Land cover factors were tested independently at site, local and landscape levels.

Table V.D.7. Results of GLM models for factors that influence bee abundance at 13 sites in Northwest Arkansas.

Dependent Variable	Independent Variable	Coefficient	*F*-stat	*df*-deviance	*df*-residual	*p*-value
Total bees	Plant genera	0.10	45.62	1	11	<0.001
Total *Bombus*	Plant genera	0.14	23.49	1	11	<0.001
B. auricomus	Plant genera	0.29	30.18	1	11	<0.001
B. fraternus	Area (ha)	0.08	51.45	1	11	<0.001
	Plant genera	0.22	84.02	1	10	<0.001
	% site developed	-5.13	9.20	1	9	0.014
B. impatiens	Plant genera	0.24	15.20	1	11	<0.01
B. pensylvanicus	Plant genera	0.11	6.90	1	11	0.022
X. virginica	% 2000m wooded	-4.53	10.77	1	11	<0.01

All models shown were significantly better at explaining between-site variability when compared to null models (significance thresholds Bonferroni- adjusted to compensate for the number of models tested in each group). Insignificant factors rejected in the model selection process are shown in the supplementary table Appendix V.H.5.

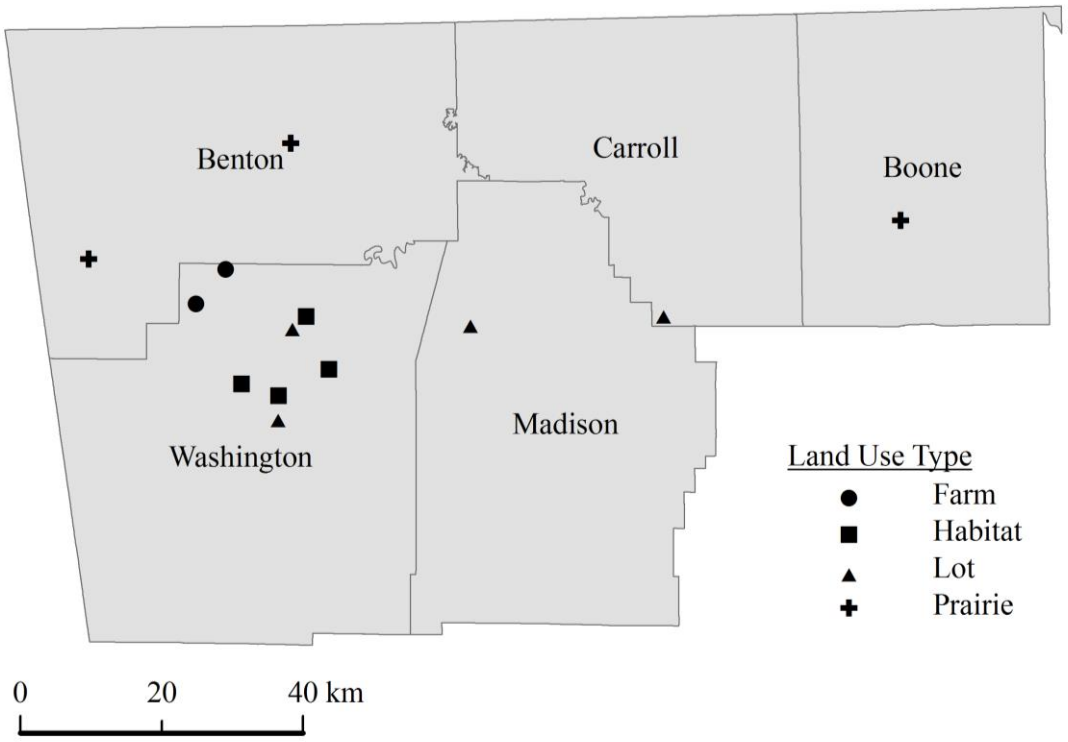


Figure V.C.1. Study locations in Northwest Arkansas, by county and site type.

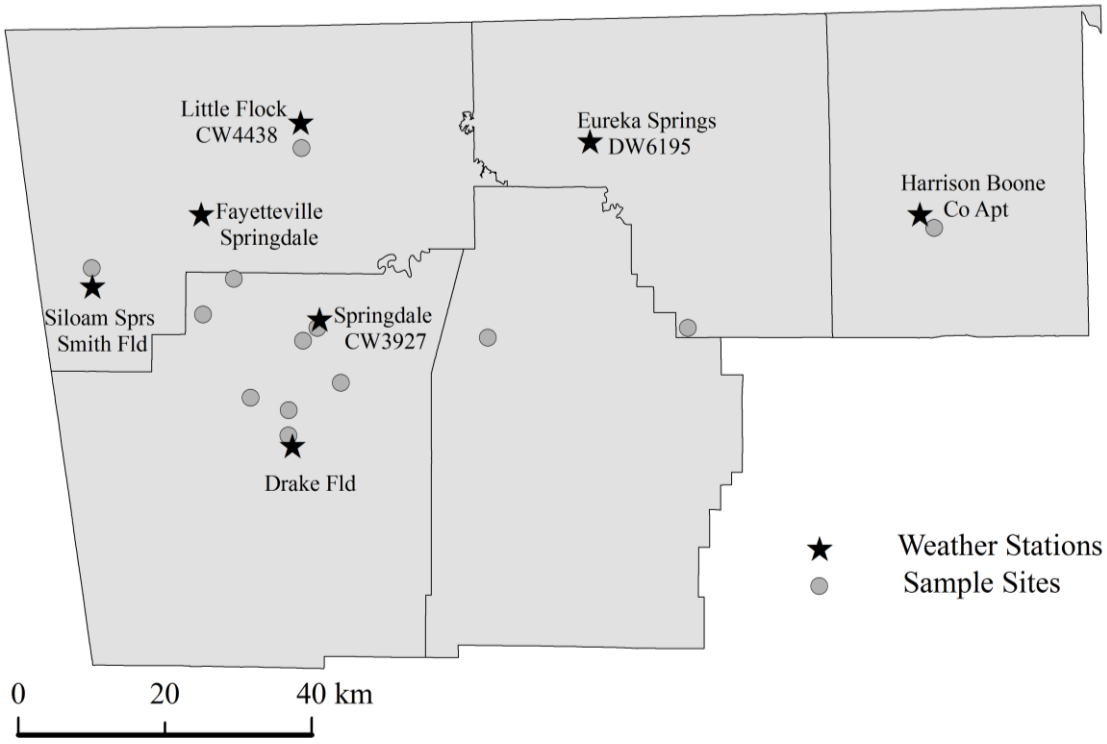


Figure V.C.2. Locations and names of weather stations (stars) that provided cumulative degree day data for each sample site (circles).

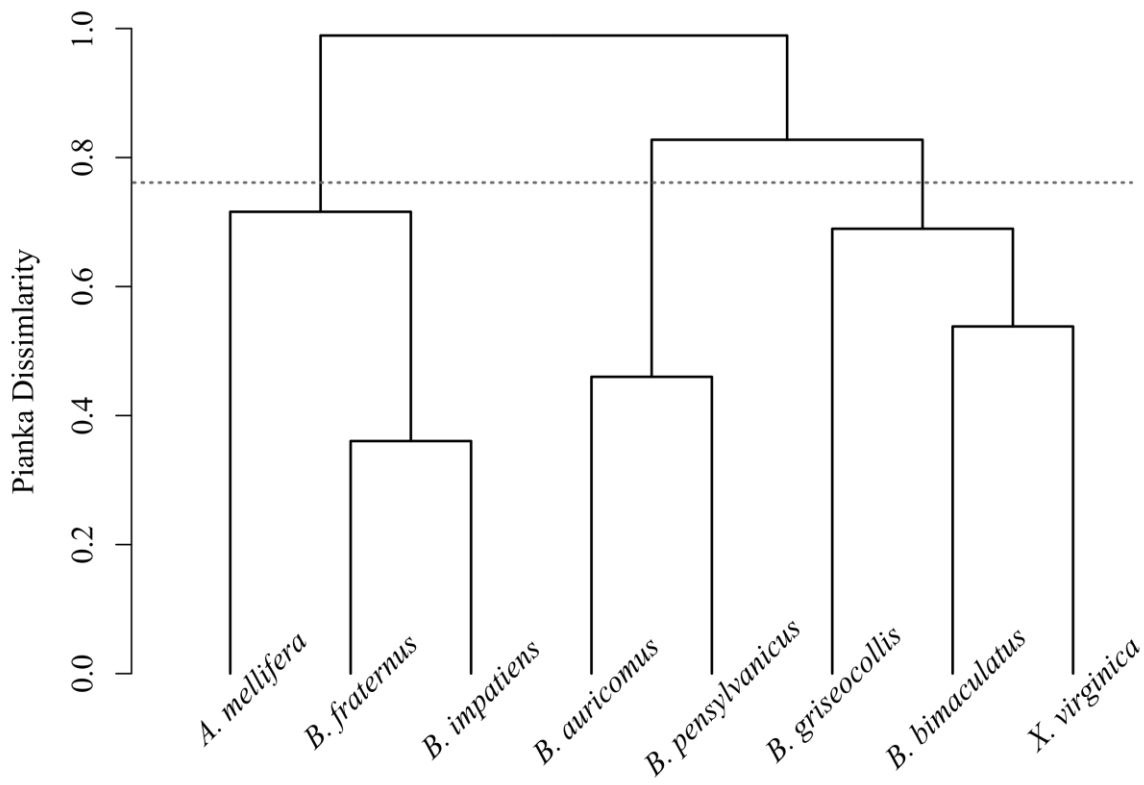


Figure V.D.1. Dendrogram illustrating cluster analysis of pairwise distances of niche overlap among species as calculated from Pianka distances (1 - O_{12}, see text). Species that cluster together are assumed to utilize similar floral resources, while those that are more distant are assumed to have less resource overlap. The dotted grey line shows the average pairwise distance among species pairs.

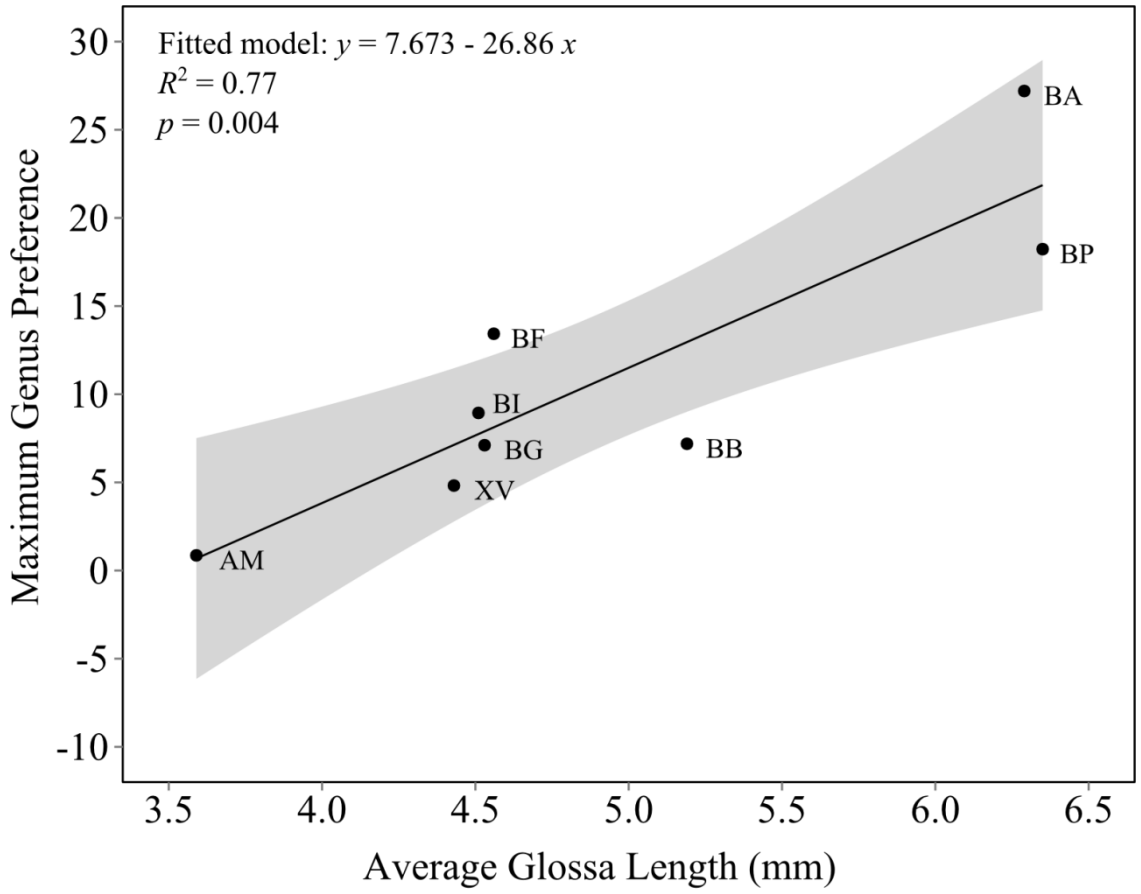


Figure V.D.2. The relationship between glossa length and maximum dietary preference at the genus level. Line shows the results of the linear regression of the equation shown,; shaded area indicates the 95% confidence interval around the regression estimates. Two letter abbreviations for each bee species are shown next to points: AM: *A. mellifera*, BA: *B. auricomus*, BB: *B. bimaculatus*, BF: *B. fraternus*, BG: *B. griseocollis*, BI: *B. impatiens*, BP: *B. pensylvanicus*, XV: *X. virginica*.

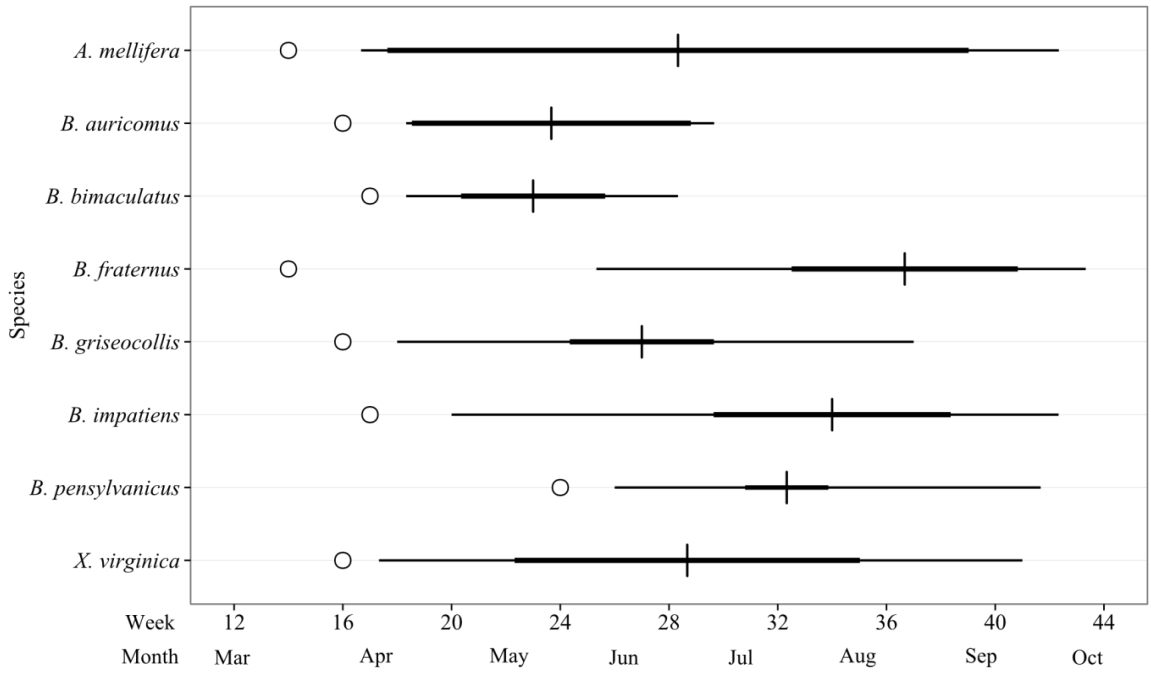


Figure V.D.3. Phenology of adult activity in bee species summarized over 2011 through 2013. Open circles represent the single earliest observation date of an active adult of each species, all of which occurred in 2012; thin lines indicate the duration of activity averaged over the three years; vertical lines indicate the average date of peak abundance, with thick lines indicating the standard deviation of this peak over the three years.

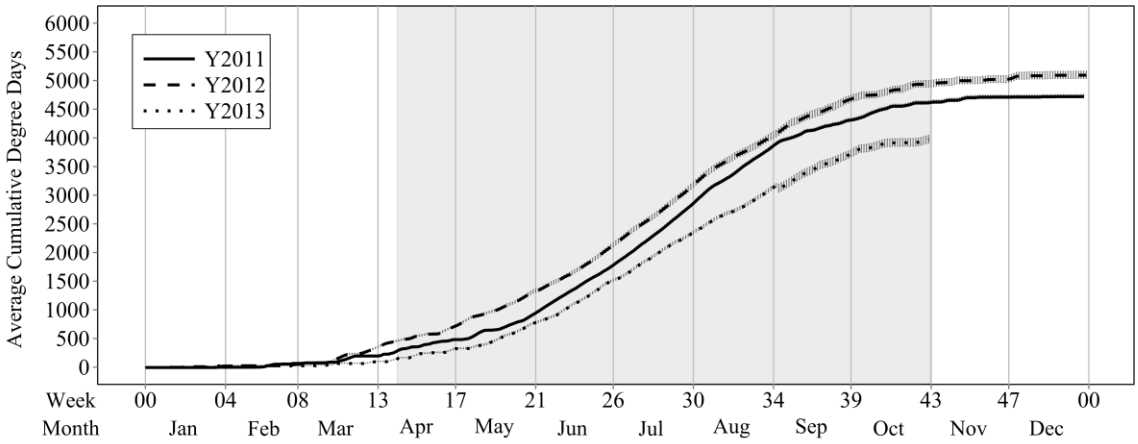


Figure V.D.4. Average cumulative degree days by sample year throughout the weeks and months of the calendar years sampled in this study. 2011: solid line, 2012: dashed line, 2013: dotted line. The shaded area indicates the general period of bee activity. Error bars on points were calculated across data from the weather stations closest to the sample locations (n=7).

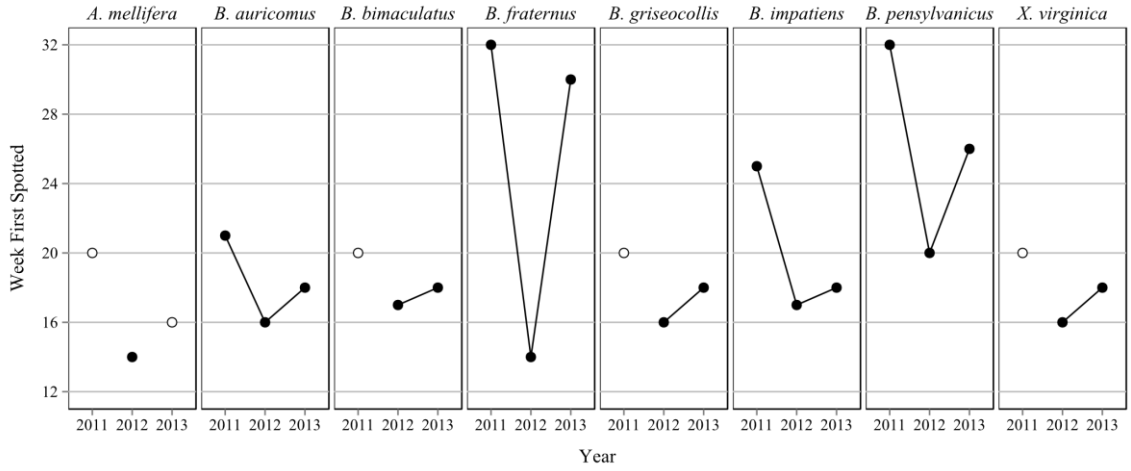


Figure V.D.5. The week of first sighting of each species by year illustrating the plasticity of bee activity start times. Open circles represent weeks that were the first week of sampling in that year (2011: week 20, 2012: week 13, 2013: week 16); closed circles represent first-sighting-weeks that were well-within the sample period that year.

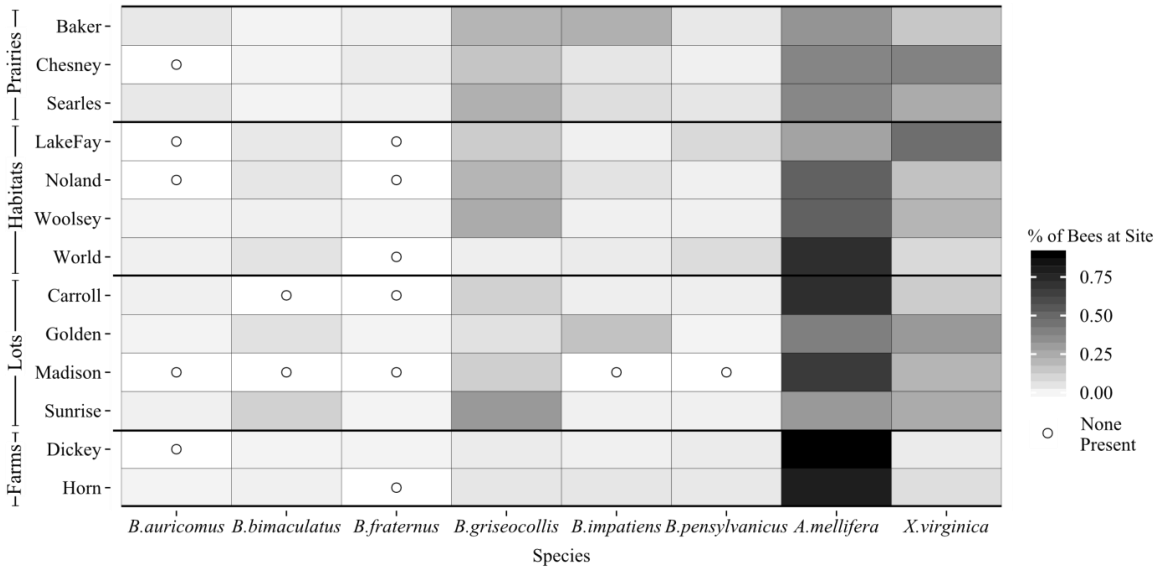


Figure V.D.6. Bee community composition at each of the 13 sites sampled in 2011–2013. The percentages of bee observations that were attributable to each species are shown along a gradient from 0.1% (light grey) to 90% (black). Open circles mark sites at which a bee species was not observed.

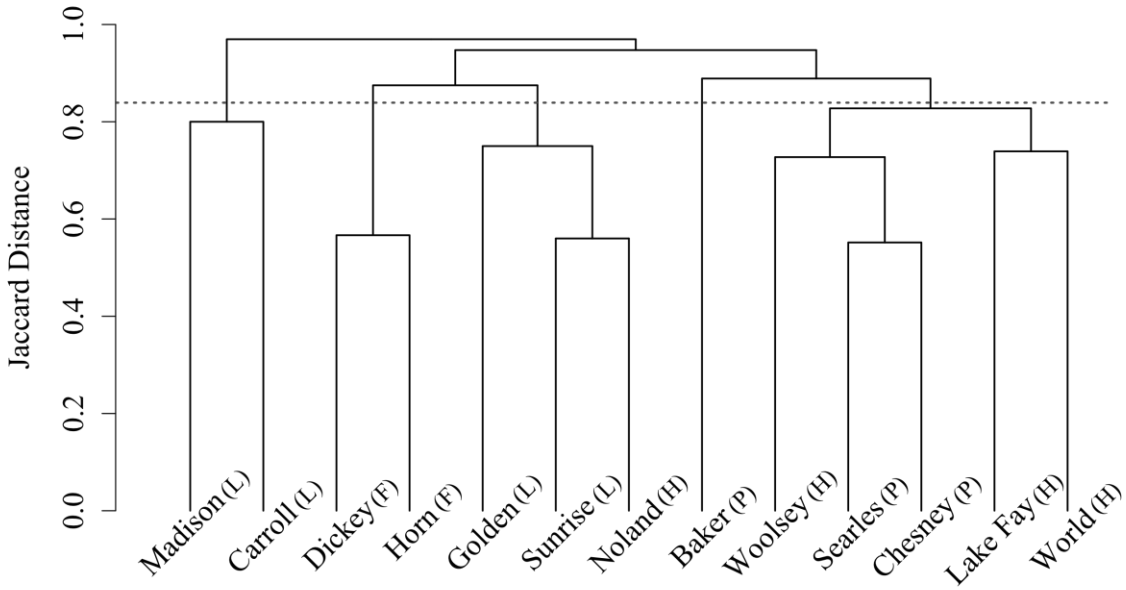


Figure V.D.7. Dendrogram of the pairwise Jaccard distance indices among site pairs based on the presence-absence of plant genera. Sites that cluster together are assumed to provide similar floral resources, while those that are more distant are assumed to provide divergent plant resources. The dotted grey line shows the average pairwise distance among site pairs. Site types are indicated by the letters F=farm, L=lot, H=habitat and P=prairie.

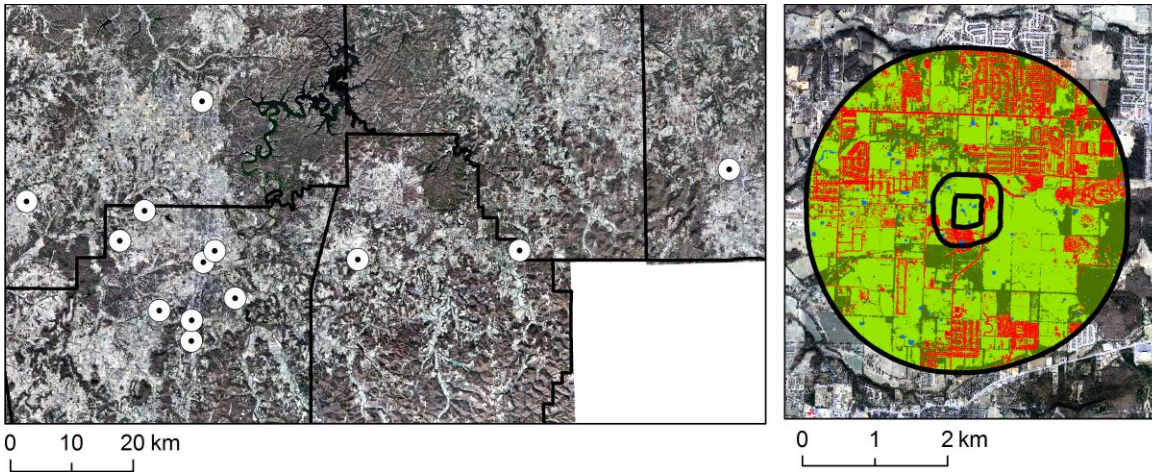


Figure V.D.8. Sample sites within the greater landscape, and an example of the land cover classification of a single site. Left: Orthographic images of the five-county sampling region (county outlines in black), with all 13 sites marked with black dots. White circles show 2 km radii surrounding each site. Right: The Woolsey site, classified and with analytical boundaries (inner: site itself, middle: 250 m=local, outer: 2,000 km=landscape) shown in black lines. Within the boundaries, red indicates developed areas, green indicates herbaceous cover and dark green indicates wooded cover.

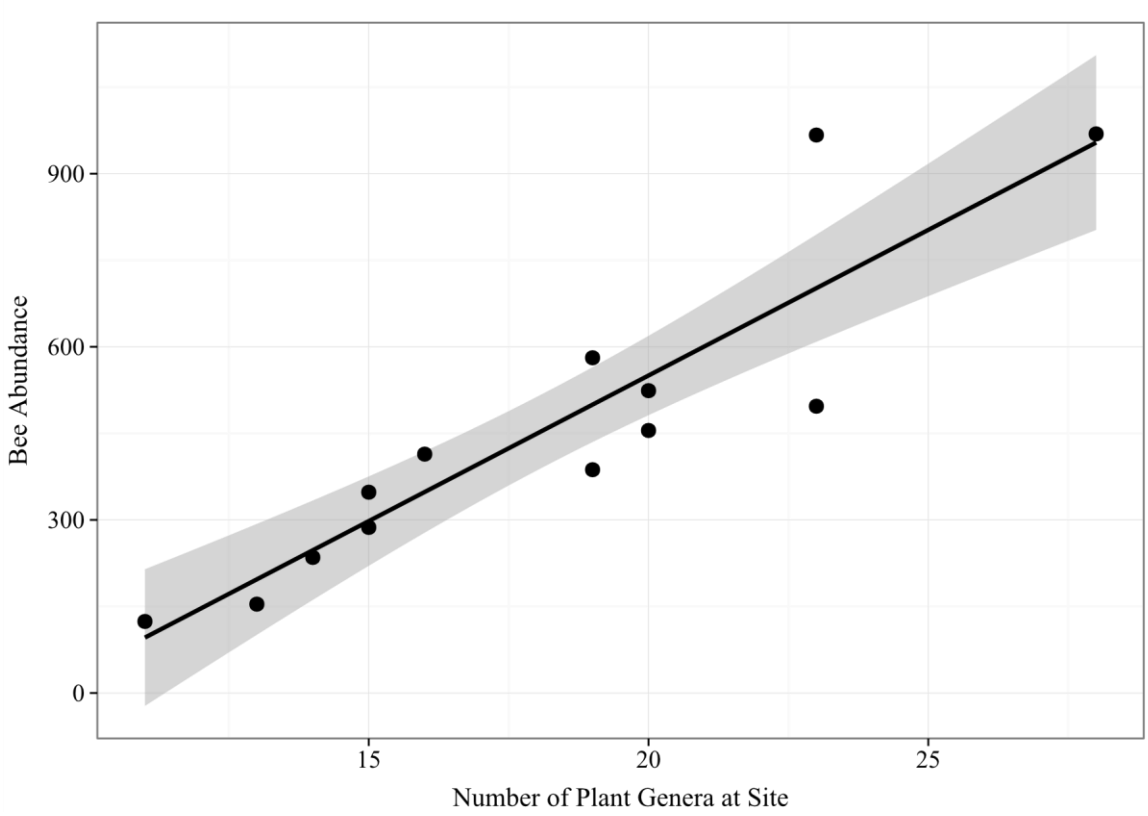


Figure V.D.9. Linear relationship between the abundance of target bees and the number of plant genera present at each site. Shaded area shows the 95% confidence interval surrounding the regression estimates. GLM results: $F_{(1,11)} = 45.62$, $p < 0.001$.

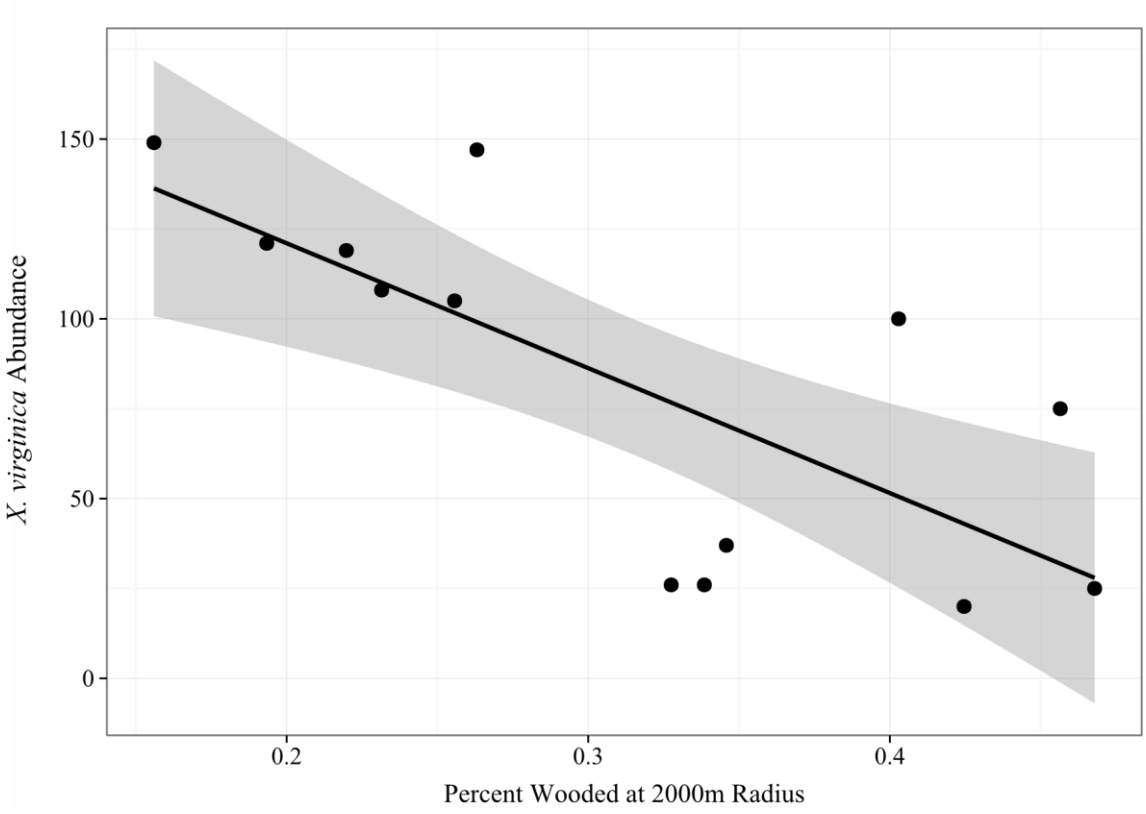


Figure V.D.10. Linear relationship between the abundance of *X. virginica* and the proportion of wooded land cover in a 2000 m radius surrounding each site. Shaded area shows the 95% confidence interval surrounding the regression estimates. GLM results: $F_{(1,11)} = 10.77$, $p < 0.01$.

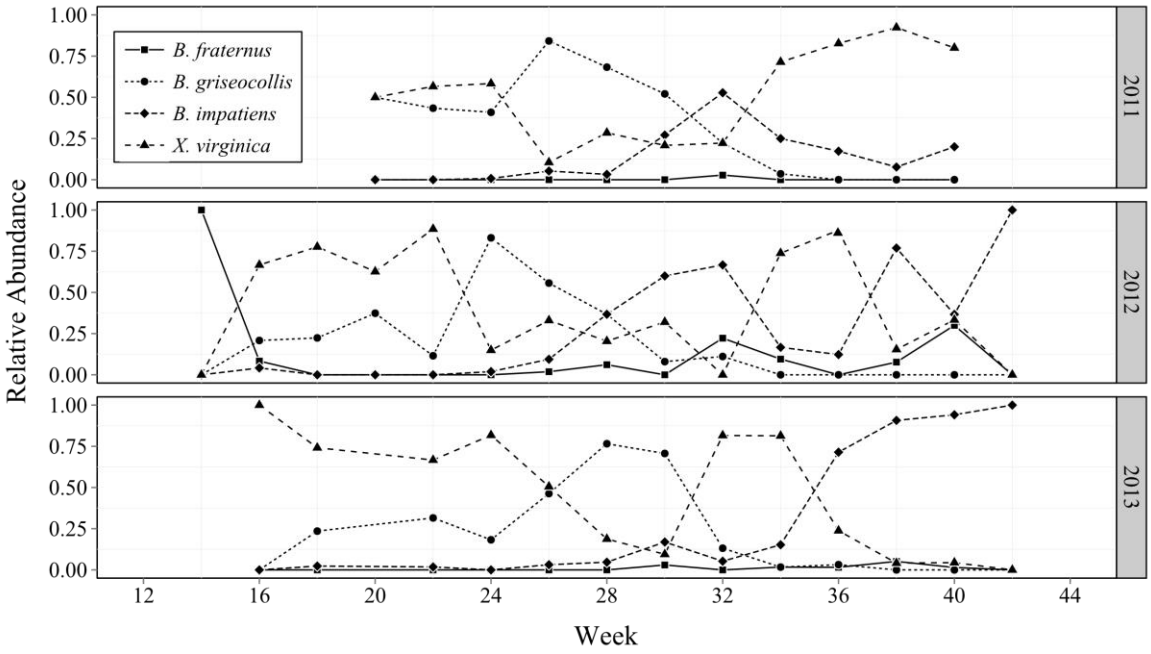


Figure V.D.11. Relative abundances of four species with overlapping glossa lengths throughout the season by week for each year.

Appendix V.H.1. Observations of floral resources used by bees during standard surveys in 2012 and 2013.

Family	Species	BA	BB	BF	BG	BI	BP	XV	AM
2012									
Amaranthaceae	*Amaranthus* sp.	0	0	0	0	0	0	0	10
Anacardiaceae	*Rhus copallinum*	0	1	0	5	0	0	0	10
Apiaceae	*Daucus carota*	0	0	0	0	0	0	0	10
	Eryngium yuccifolium	0	0	1	0	2	0	0	0
	Zizia aptera	0	0	1	1	0	0	0	0
Apocynaceae	*Asclepias hirtella*	0	0	0	5	0	0	0	0
	Asclepias viridis	0	0	0	10	0	0	18	0
Asparagaceae	*Asparagus officinalis*	0	1	0	0	0	0	0	3
Asteraceae	*Bidens aristosa*	0	0	0	0	0	0	8	1
	Carduus nutans	0	1	0	24	0	0	1	0
	Centaurea americana	0	0	0	1	0	0	0	0
	Centaurea stoebe	0	0	0	1	0	0	2	10
	Cichorium intybus	0	0	0	0	0	0	0	5
	Cirsium discolor	0	0	0	0	0	1	0	0
	Cirsium vulgare	0	0	0	1	10	0	0	3
	Elephantopus carolinianus	0	0	0	0	0	1	0	2
	Eupatorium serotinum	0	0	0	0	0	0	16	5
	Eupatorium sp.	0	0	0	0	0	0	1	0
	Grindelia lanceolata	0	0	0	0	0	0	0	42
	Helianthus mollis	0	0	1	0	3	0	0	3
	Liatris pycnostachya	0	0	0	0	0	1	0	0
	Liatris sp.	0	0	0	2	0	0	2	2
	Rudbeckia hirta	0	0	0	0	0	0	0	1
	Silphium integrifolium	0	0	4	0	1	5	9	7
	Silphium laciniatum	0	0	0	2	0	0	0	2
	Silphium perfoliatum	0	0	0	0	2	0	0	6
	Silphium sp.	0	0	3	1	0	1	0	0
	Solidago sp.	0	0	4	0	6	0	2	16
	Symphyotrichum sp. Blue	0	0	0	0	4	0	0	2
	Symphyotrichum sp. White	0	0	0	0	0	0	0	4
	Taraxacum officinale	0	0	0	0	0	0	0	1
	Verbesina virginica	0	0	1	0	8	0	4	35
	Vernonia sp.	0	0	0	0	0	14	1	1
	Unidentified thistle	0	5	0	1	2	5	1	1
Brassicaceae	*Brassica rapa*	0	0	0	0	0	0	2	0
Caprifoliaceae	*Lonicera japonica*	1	0	0	1	0	0	4	7
Commelinaceae	*Tradescantia* sp.	0	0	0	0	0	0	0	3

Appendix V.H.1. (Cont.)

Family	Species	BA	BB	BF	BG	BI	BP	XV	AM
2012 (Cont.)									
Convolvulaceae	*Ipomoea hederacea*	0	0	0	0	2	2	0	3
Cucurbitaceae	*Citrullus lanatus*	0	0	0	0	1	0	1	18
Cucurbitaceae	*Cucumis melo*	0	0	0	0	0	0	0	21
	Cucumis sativus	0	0	0	0	1	0	0	31
	Cucumis sp.	0	0	0	0	1	0	0	4
	Cucurbita pepo	0	0	0	0	0	0	0	4
Fabaceae	*Baptisia alba*	4	0	0	2	0	15	0	0
	Baptisia bracteata	1	0	0	0	0	0	0	0
	Melilotus officinalis	0	0	0	0	0	0	1	15
	Mimosa nuttallii	0	0	0	0	0	0	1	12
	Trifolium incarnatum	0	0	0	0	0	0	0	2
	Trifolium pratense	1	0	0	0	0	1	3	0
	Trifolium repens	0	0	0	1	0	0	1	326
	Vicia villosa	2	23	0	22	1	5	43	21
	Vigna unguiculata	0	0	0	0	0	4	1	8
Lamiaceae	*Monarda fistulosa*	21	0	0	19	0	8	1	0
	Physostegia angustifolia	1	0	0	1	3	0	28	17
	Physostegia virginiana	0	0	0	0	0	0	1	0
	Pycnanthemum pilosum	0	0	1	5	22	1	1	75
	Pycnanthemum tenuifolium	3	3	0	37	2	0	3	38
	Salvia azurea	0	0	0	0	7	2	7	11
	Teucrium canadense	0	2	0	14	1	6	25	3
Liliaceae	*Camassia angusta*	0	0	0	0	0	0	6	4
Malvaceae	*Abelmoschus esculentus*	0	0	0	0	9	7	3	32
Onagraceae	*Oenothera speciosa*	0	0	0	0	0	0	0	1
Oxalidaceae	*Oxalis* sp.	0	0	0	0	0	0	0	3
Passifloraceae	*Passiflora incarnata*	0	0	5	0	0	2	67	31
Plantaginaceae	*Penstemon digitalis*	4	5	0	0	0	0	13	15
	Penstemon sp.	0	0	0	0	0	0	1	0
Polygonaceae	*Persicaria* sp.	0	0	0	0	0	0	0	52
Primulaceae	*Dodecatheon meadia*	0	0	1	0	0	0	0	0
Rhamnaceae	*Ceanothus herbaceus*	0	0	0	2	0	0	2	1
Rosaceae	*Rosa* sp.	0	1	0	11	0	0	19	8
	Rubus sp.	0	0	0	3	0	0	3	0
Rubiaceae	*Cephalanthus occidentalis*	0	1	0	59	0	0	0	2
Scrophulariaceae	*Veronicastrum virginicum*	0	0	1	5	0	0	0	2
Solanaceae	*Physalis* sp.	0	0	0	0	1	0	0	58
	Solanum carolinense	0	0	0	3	2	7	0	1

Appendix V.H.1. (Cont.)

Family	Species	BA	BB	BF	BG	BI	BP	XV	AM
2012 (Cont.)									
Solanaceae (Cont.)	*Solanum lycopersicum*	0	0	0	0	0	0	1	0
2013									
Acanthaceae	*Ruellia humilis*	0	0	0	0	0	0	1	0
Apiaceae	*Daucus carota*	0	0	0	0	0	0	0	2
Apiaceae (Cont.)	*Eryngium yuccifolium*	0	0	0	0	3	0	2	0
	Torilis arvensis	0	0	0	0	0	0	0	2
	Zizia aptera	0	0	0	0	0	0	0	1
Apocynaceae	*Apocynum cannabinum*	0	0	0	0	0	0	0	14
	Asclepias hirtella	0	0	1	27	1	0	1	0
	Asclepias incarnata	0	0	0	0	0	1	2	0
	Asclepias viridis	0	0	0	19	0	0	16	1
Asteraceae	*Bidens aristosa*	0	0	2	1	2	0	0	0
	Carduus nutans	0	1	0	9	0	1	1	0
	Centaurea stoebe	1	1	1	18	0	0	2	26
	Cichorium intybus	0	0	0	0	0	0	1	1
	Cirsium altissimum	0	0	0	0	0	1	0	0
	Cirsium discolor	0	0	0	0	0	5	0	0
	Cirsium vulgare	0	0	0	1	0	0	1	1
	Conyza canadensis	0	0	0	0	0	0	1	0
	Coreopsis sp.	0	0	0	0	0	0	0	1
	Dipsacus fullonum	0	0	0	12	0	0	0	1
	Echinacea pallida	0	0	0	4	0	0	0	5
	Echinacea purpurea	0	0	0	1	0	0	0	3
	Elephantopus carolinianus	0	0	0	0	0	0	0	2
	Helenium flexuosum	0	0	0	0	6	0	0	0
	Helianthus grosseserratus	0	0	0	0	0	0	1	1
	Helianthus maximilianii	0	0	0	0	2	0	0	0
	Helianthus mollis	0	0	0	1	0	2	0	0
	Leucanthemum vulgare	0	0	0	0	0	0	2	0
	Liatris pycnostachya	0	0	3	37	0	0	3	17
	Rudbeckia hirta	0	0	0	2	1	0	1	9
	Rudbeckia sp.	0	0	0	0	0	0	1	0
	Silphium integrifolium	1	0	0	18	26	0	1	59
	Silphium laciniatum	0	0	0	0	1	0	0	2
	Silphium perfoliatum	0	0	0	2	1	2	1	29
	Solidago altissima	0	0	2	0	12	0	0	10
	Solidago canadensis	0	0	0	0	1	0	3	10
	Solidago radula	0	0	0	0	0	0	0	3

Appendix V.H.1. (Cont.)

Family	Species	BA	BB	BF	BG	BI	BP	XV	AM
2013 (Cont.)									
Asteraceae (Cont.)	*Solidago rigida*	0	0	0	0	1	0	0	0
	Solidago rugosa	0	0	0	0	1	0	0	0
	Solidago sp.	0	0	0	0	15	0	0	20
	Solidago speciosa	0	0	0	0	68	0	0	8
	Symphyotrichum sp. Blue	0	0	0	0	14	0	0	21
	Symphyotrichum sp. White	0	0	1	0	24	0	1	102
	Verbesina alternifolia	0	0	0	1	23	0	7	6
	Verbesina helianthoides	0	0	0	1	0	0	12	34
	Verbesina virginica	0	0	1	0	11	0	1	39
	Vernonia arkansana	0	0	0	0	0	2	1	1
	Vernonia baldwinii	0	0	0	0	0	0	4	2
Brassicaceae	*Brassica oleracea*	0	0	0	1	0	0	2	12
	Brassica rapa	0	0	0	0	0	0	0	61
	Brassica sp.	0	0	0	0	0	0	8	10
	Eruca sativa	0	0	0	0	0	0	0	1
Caprifoliaceae	*Lonicera japonica*	0	0	0	0	0	0	0	9
Cucurbitaceae	*Citrullus lanatus*	0	0	0	0	0	0	0	19
	Cucumis melo	0	0	0	0	0	0	0	34
	Cucumis sativus	0	0	0	2	0	0	0	30
	Cucurbita pepo	0	0	0	0	1	0	0	0
Fabaceae	*Albizia julibrissin*	0	0	0	1	0	0	0	1
	Baptisia alba	4	0	0	0	0	1	0	0
	Baptisia bracteata	3	0	0	0	0	0	0	0
	Cercis canadensis	0	0	0	0	1	0	0	0
	Dalea candida	0	0	0	1	0	0	3	0
	Desmodium panciulatum	0	0	0	0	10	0	0	0
	Desmodium sp.	0	0	0	0	0	1	0	0
	Lathyrus latifolius	0	0	0	0	0	0	1	0
	Lespedeza violacea	0	0	0	0	0	0	0	2
	Melilotus officinalis	0	0	0	9	0	0	0	49
	Mimosa nuttallii	0	0	0	0	1	0	0	6
	Tephrosia virginiana	0	0	0	0	0	0	6	0
	Trifolium incarnatum	0	0	0	4	0	0	2	45
	Trifolium pratense	0	0	0	0	0	2	0	50
	Trifolium repens	0	0	0	0	1	0	0	15
	Vicia sativa	0	21	0	12	1	0	36	79
	Vicia villosa	0	0	0	0	0	0	0	1
	Vigna unguiculata	0	0	0	1	0	0	0	0

Appendix V.H.1. (Cont.)

Family	Species	BA	BB	BF	BG	BI	BP	XV	AM
2013 (Cont.)									
Gentianaceae	*Gentiana puberulenta*	0	0	0	0	1	2	0	0
Lamiaceae	*Lamium purpureum*	0	1	0	6	1	0	4	14
	Monarda citriodora	0	2	0	10	0	0	6	0
	Monarda fistulosa	0	2	0	0	0	3	2	0
	Perilla frutescens	0	0	0	0	1	0	0	17
	Physostegia angustifolia	1	1	0	1	1	0	73	17
	Pycnanthemum pilosum	0	0	0	1	1	0	1	29
	Pycnanthemum tenuifolium	0	0	0	18	0	0	20	121
Lamiaceae (Cont.)	*Salvia azurea*	0	0	0	0	4	4	11	12
	Teucrium canadense	0	2	0	25	3	6	7	4
Liliaceae	*Nothoscordum bivalve*	0	0	0	0	0	0	0	5
Lythraceae	*Lythrum alatum*	0	0	0	0	0	0	0	2
Onagraceae	*Gaura* sp.	0	0	0	0	0	0	0	1
	Oenothera speciosa	0	0	0	0	0	0	0	3
Papaveraceae	*Corydalis* sp.	0	0	0	0	0	0	12	5
Passifloraceae	*Passiflora incarnata*	0	0	1	0	0	0	53	8
Plantaginaceae	*Penstemon digitalis*	0	0	0	0	0	0	36	3
Primulaceae	*Dodecatheon meadia*	0	0	0	1	0	0	0	0
Ranunculaceae	*Delphinium carolinianum*	2	0	0	0	0	0	0	0
	Ranunculus sp.	0	0	0	0	0	0	0	1
Rhamnaceae	*Ceanothus herbaceus*	0	0	0	0	0	0	1	0
Rosaceae	*Fragaria* sp.	0	0	0	0	0	0	0	22
	Malus sp.	0	0	0	0	0	0	0	6
	Rosa sp.	0	0	0	1	1	0	0	3
Rubiaceae	*Cephalanthus occidentalis*	1	0	2	35	1	1	4	4
Scrophulariaceae	*Pedicularis canadensis*	0	0	0	8	0	0	14	0
	Verbascum blattaria	0	0	0	0	0	0	1	13
Solanaceae	*Physalis* sp.	0	0	0	0	0	0	0	26
	Solanum carolinense	0	0	0	1	0	0	0	0
Verbenaceae	*Verbena hastata*	0	0	0	0	1	1	0	0

BA = *Bombus auricomus*, BB = *B. bimaculatus*, BF = *B. fraternus*, BG = *B. griseocollis*, BI = *B. impatiens*, BP = *B. pensylvanicus*, XV= *Xylocopa virginica*, AM = *Apis mellifera*

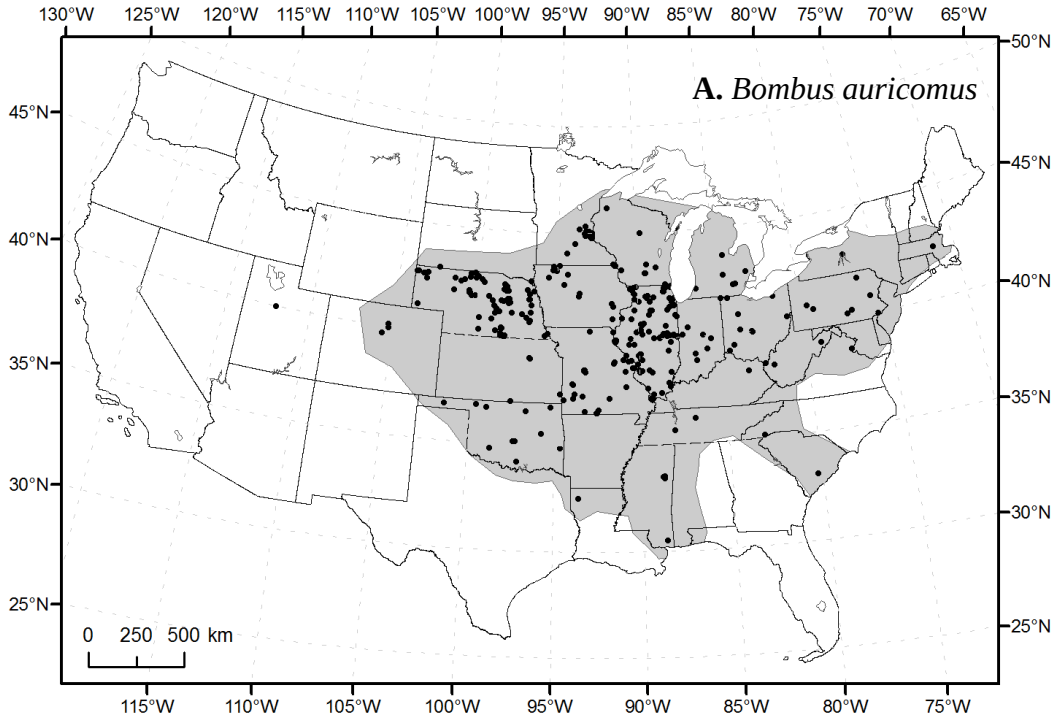
APPENDIX V.H.2. The top eight floral resources used by each bee species during standard surveys in Northwest Arkansas in 2012 and 2013.

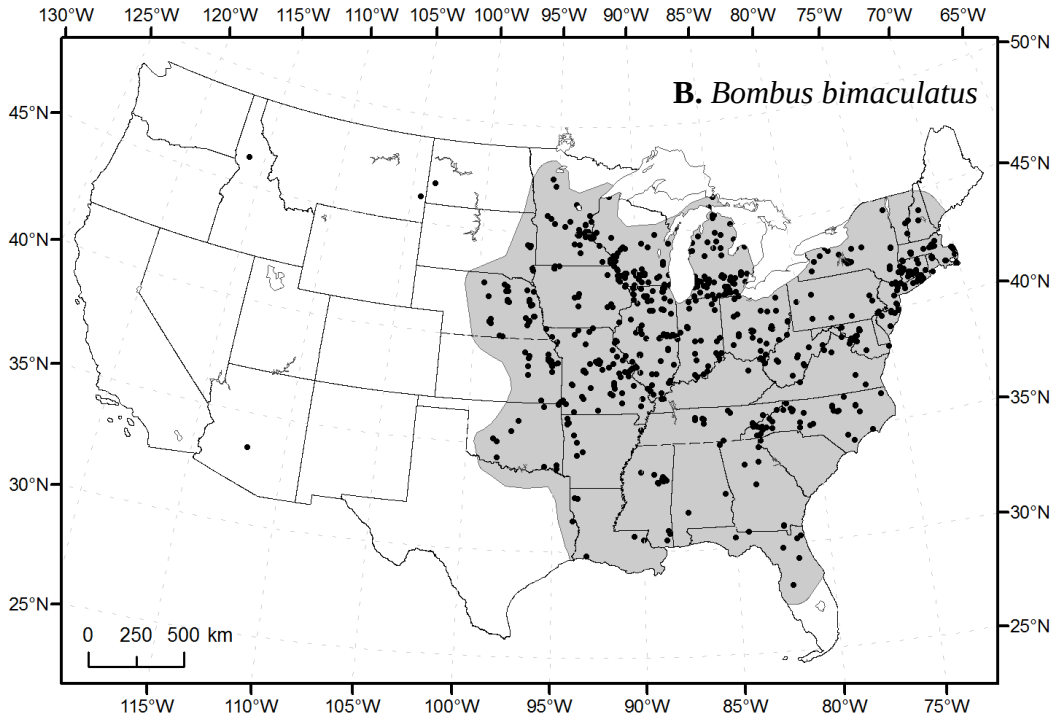
Bee Species	Plant Species	% of Observations
B. auricomus	*Monarda fistulosa*	41.2%
	Baptisia alba	15.7%
	Baptisia bracteata	7.8%
	Penstemon digitalis	7.8%
	Pycnanthemum tenuifolium	5.9%
	Delphinium carolinianum	3.9%
	Physostegia angustifolia	3.9%
	Vicia villosa	3.9%
B. bimaculatus	*Vicia villosa*	33.3%
	Vicia sativa	30.4%
	Penstemon digitalis	7.2%
	Teucrium canadense	5.8%
	Pycnanthemum tenuifolium	4.3%
	Carduus nutans	2.9%
	Monarda citriodora	2.9%
	Monarda fistulosa	2.9%
B. fraternus	*Passiflora incarnata*	16.2%
	Silphium integrifolium	10.8%
	Solidago sp.	10.8%
	Liatris pycnostachya	8.1%
	Silphium sp.	8.1%
	Bidens aristosa	5.4%
	Cephalanthus occidentalis	5.4%
	Solidago altissima	5.4%
	Verbesina virginica	5.4%
B. griseocollis	*Cephalanthus occidentalis*	17.7%
	Pycnanthemum tenuifolium	10.4%
	Teucrium canadense	7.4%
	Liatris pycnostachya	7.0%
	Carduus nutans	6.2%
	Asclepias hirtella	6.0%
	Asclepias viridis	5.5%
	Vicia villosa	4.2%

APPENDIX V.H.2. (Cont.)

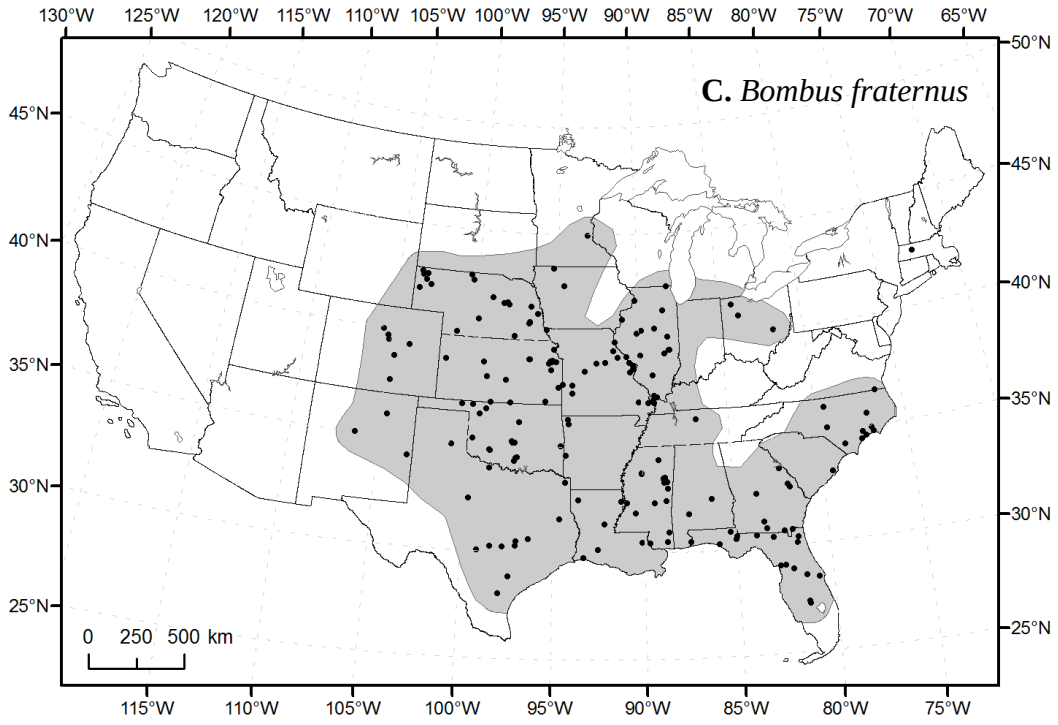
Bee Species	Plant Species	% of Observations
B. impatiens	*Solidago speciosa*	20.5%
	Symphyotrichum sp.	12.7%
	Silphium integrifolium	8.1%
	Pycnanthemum pilosum	6.9%
	Verbesina alternifolia	6.9%
	Solidago sp.	6.3%
	Verbesina virginica	5.7%
	Solidago altissima	3.6%
B. pensylvanicus	*Baptisia alba*	13.6%
	Vernonia sp.	11.9%
	Teucrium canadense	10.2%
	Monarda fistulosa	9.3%
	Abelmoschus esculentus	5.9%
	Solanum carolinense	5.9%
	Cirsium discolor	5.1%
	Salvia azurea	5.1%
A. mellifera	*Trifolium repens*	15.6%
	Pycnanthemum tenuifolium	7.3%
	Symphyotrichum sp. White	4.9%
	Pycnanthemum pilosum	4.8%
	Physalis sp.	3.8%
	Vicia sativa	3.6%
	Verbesina virginica	3.4%
	Silphium integrifolium	3.0%
X. virginica	*Passiflora incarnata*	17.9%
	Physostegia angustifolia	15.0%
	Penstemon digitalis	7.3%
	Vicia villosa	6.4%
	Vicia sativa	5.4%
	Asclepias viridis	5.1%
	Teucrium canadense	4.8%
	Pycnanthemum tenuifolium	3.4%

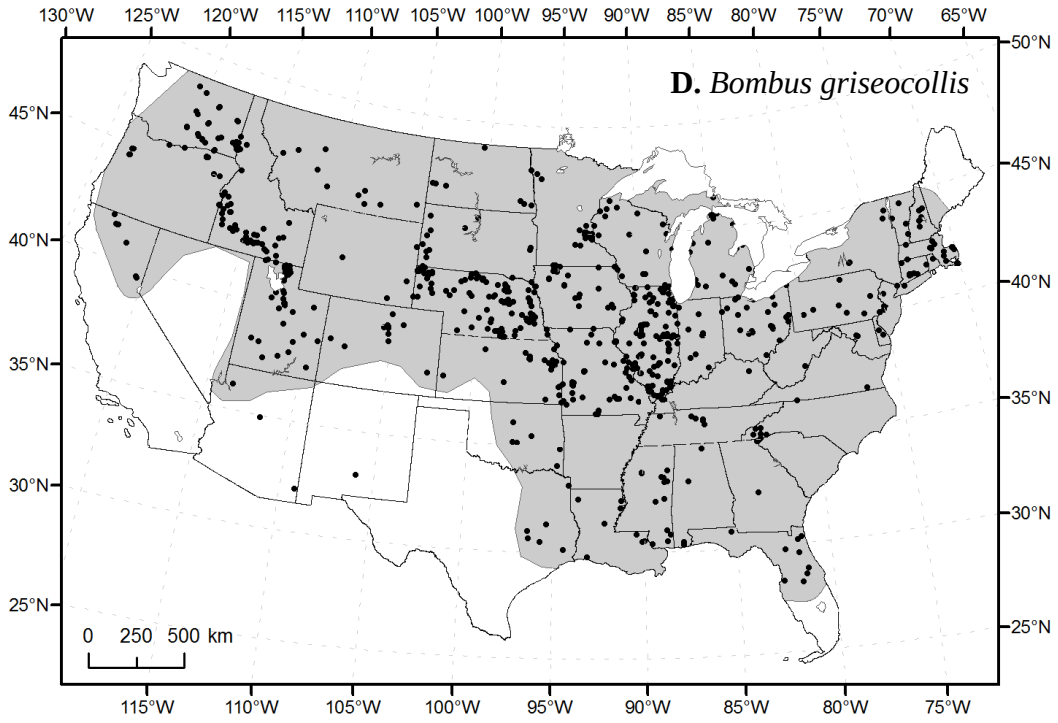
APPENDIX V.H.3. Distribution within the United States of all species in this study, except *A. mellifera*.



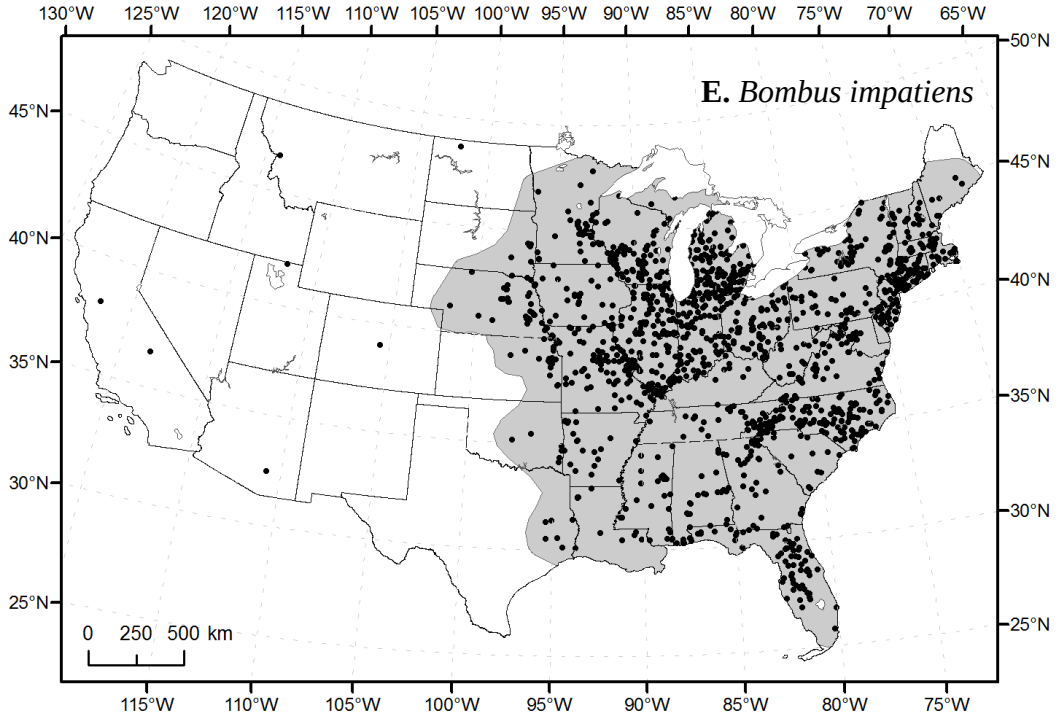


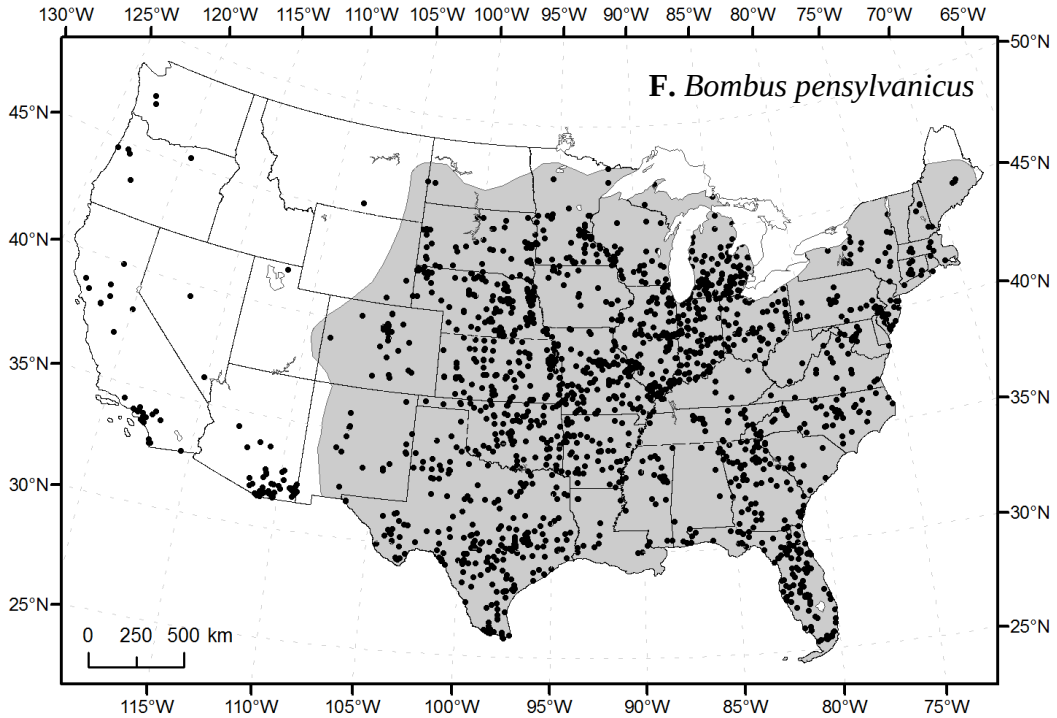
APPENDIX V.H.3. (Cont.)



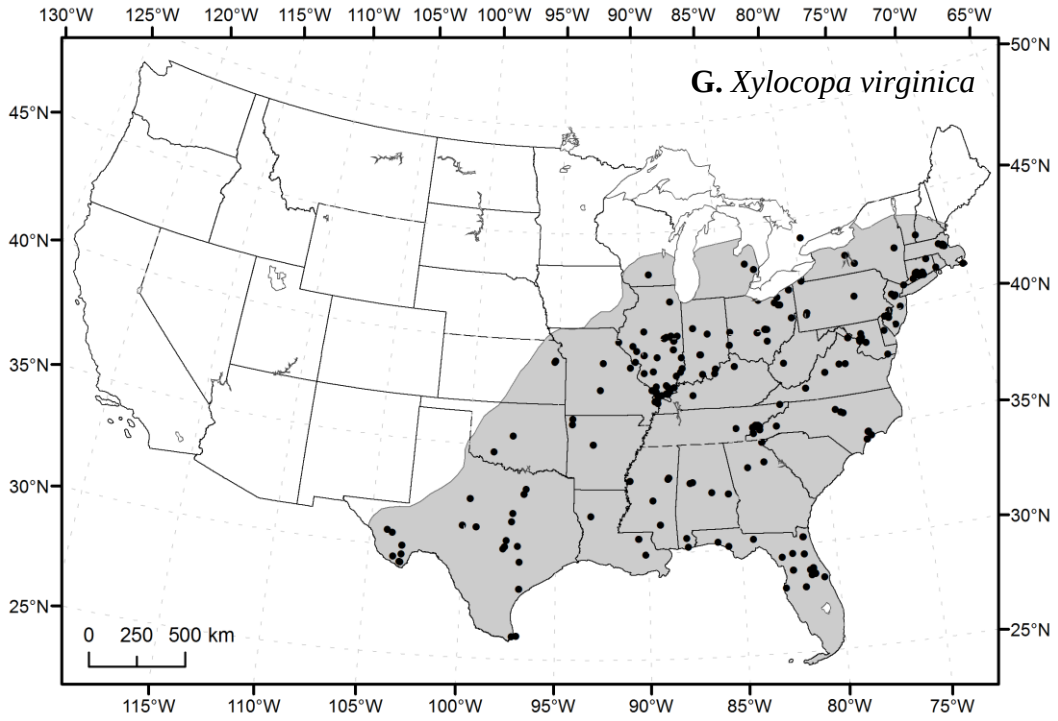


APPENDIX V.H.3. (Cont.)





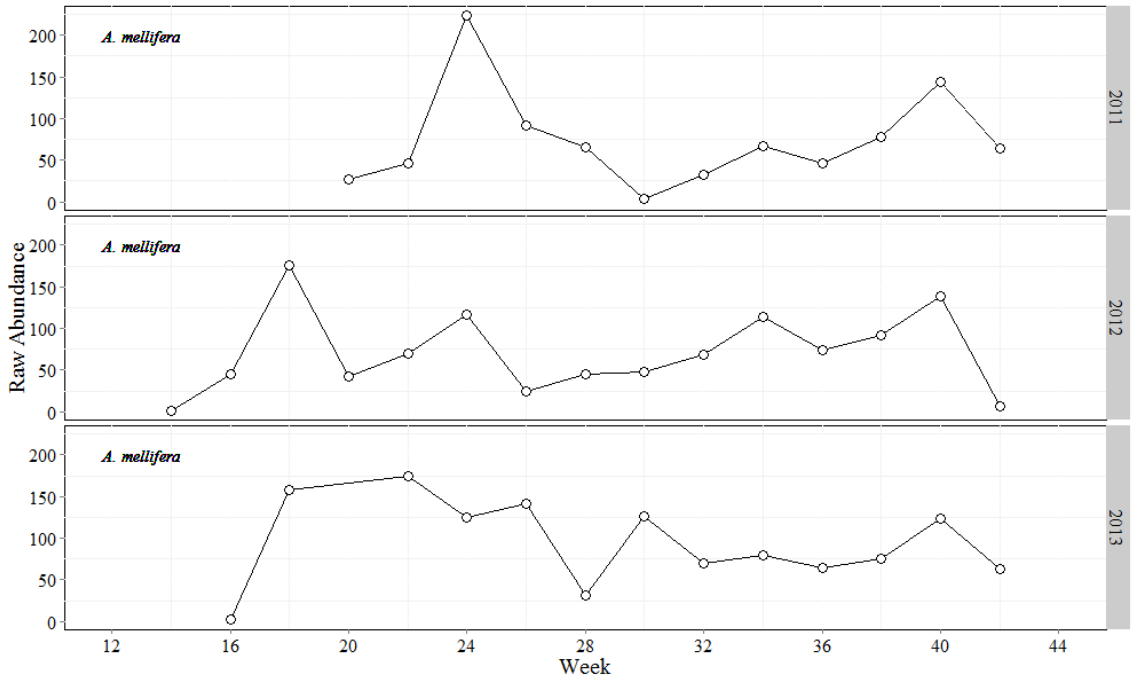
APPENDIX V.H.3. (Cont.)

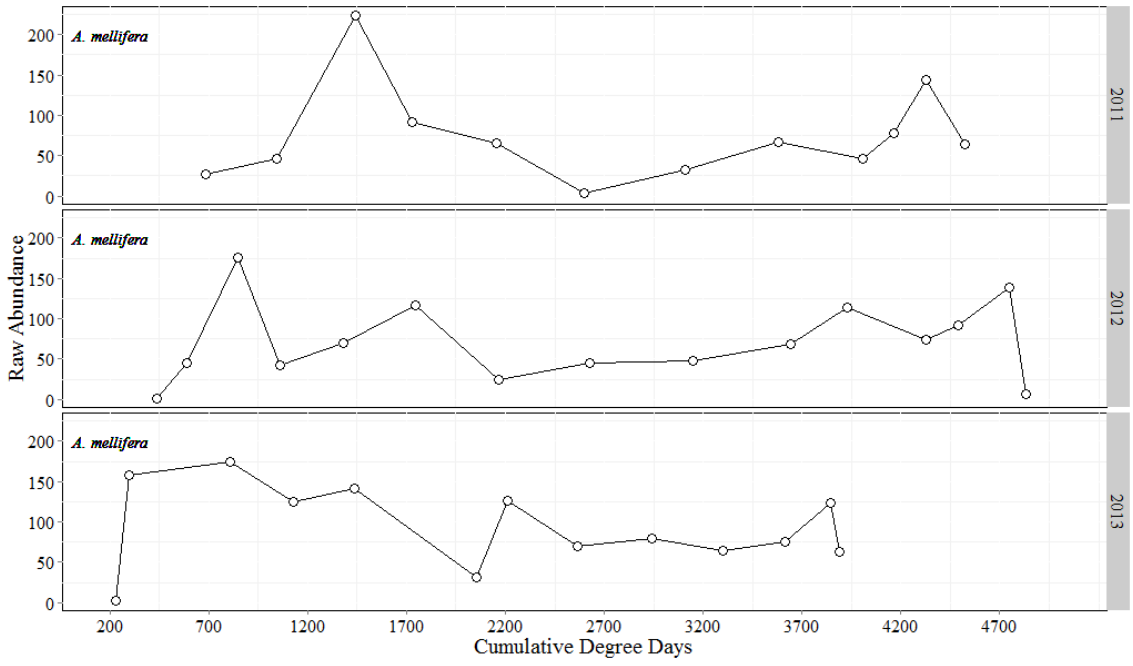


A. *Bombus auricomus*
B. *B. bimaculatus*
C. *B. fraternus*
D. *B. griseocollis*
E. *B. impatiens*
F. *B. pensylvanicus* (range in grey excludes *B. pensylvanicus sonorous*)
G. *Xylocopa virginica*

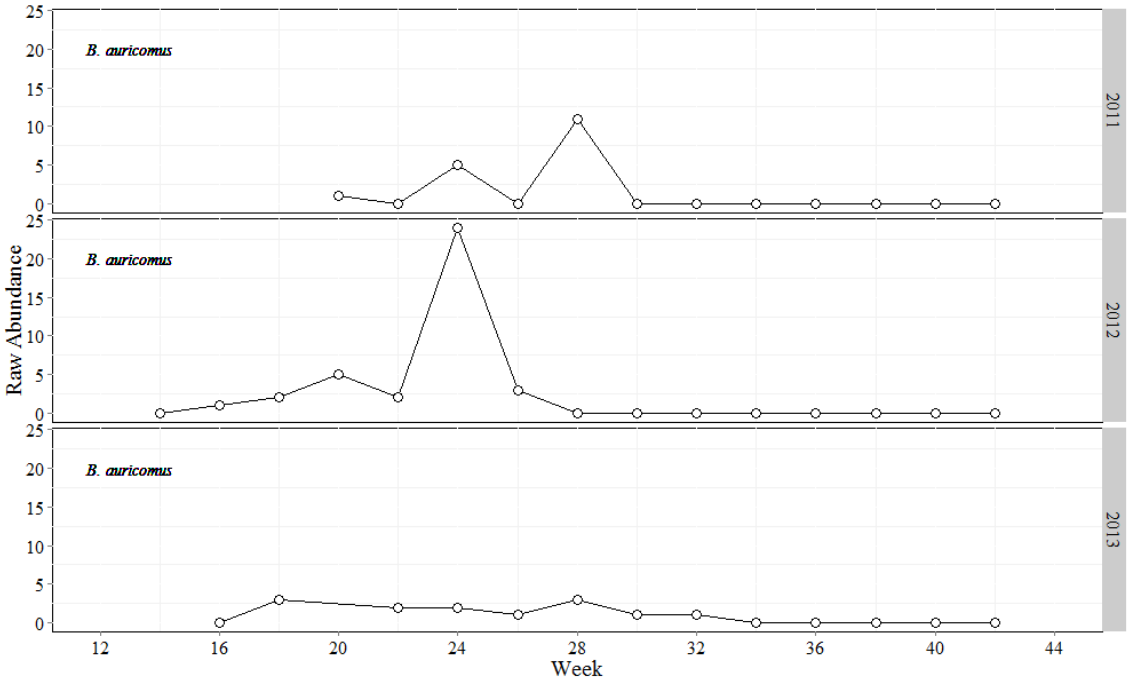
Maps were constructed from records obtained from GBIF (black dots). Range estimates shown in grey. See text for details.

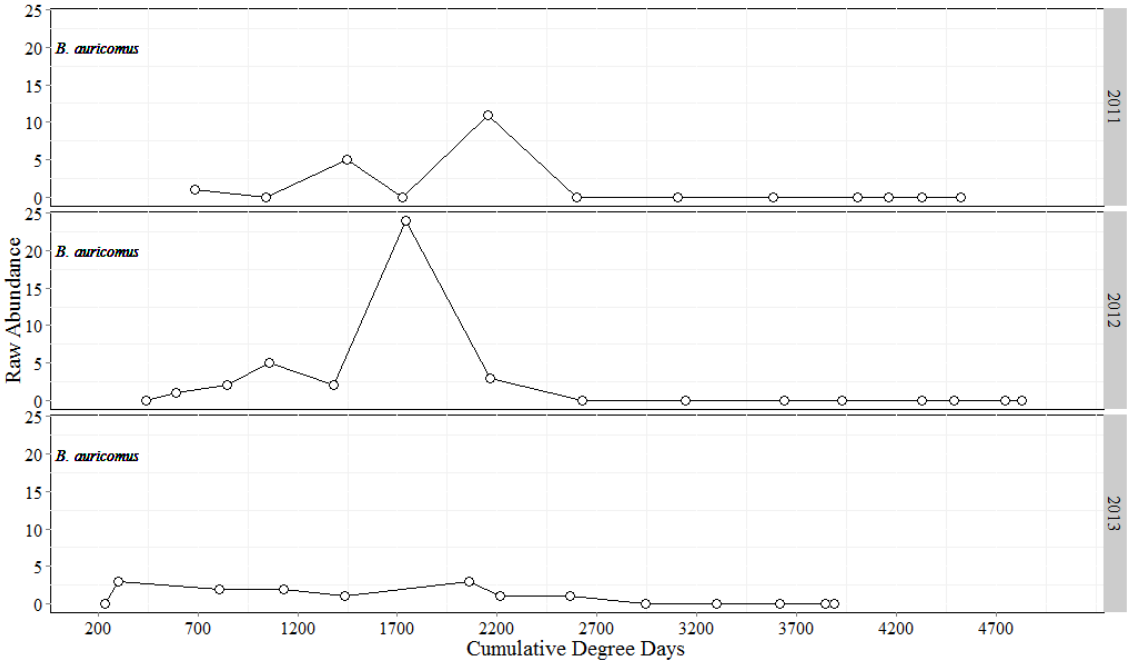
APPENDIX V.H.4. Phenology of the raw abundances of adults of each species in each year by week (upper) and cumulative degree days since January 1 of each year (lower).





APPENDIX V.H.4. (Cont.)





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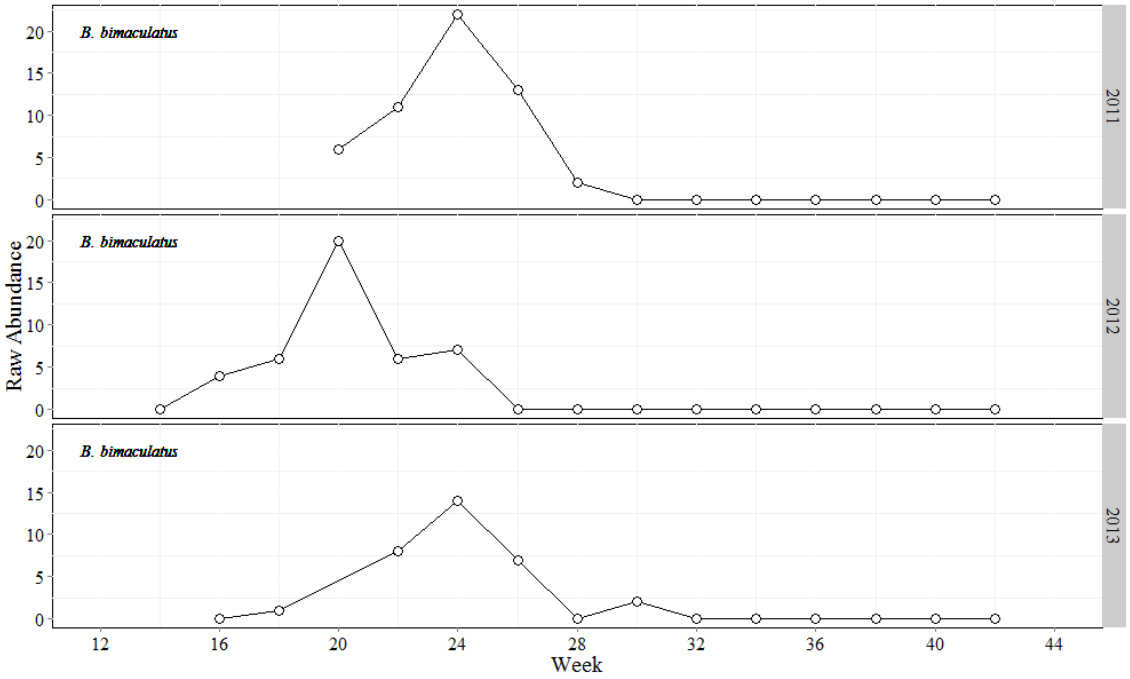

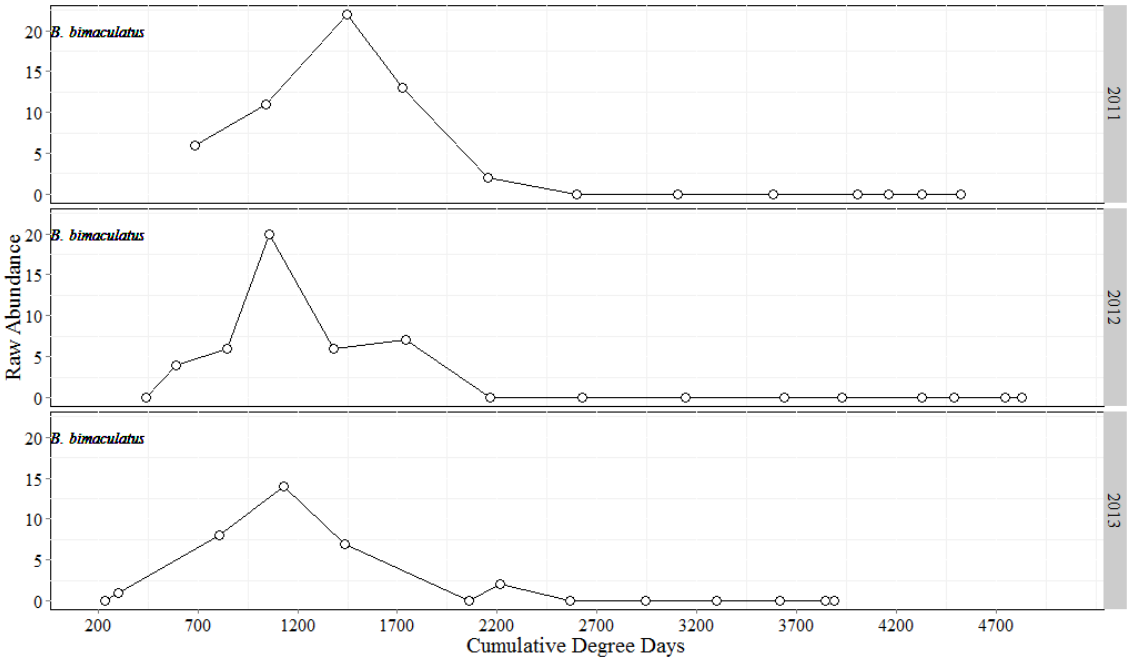

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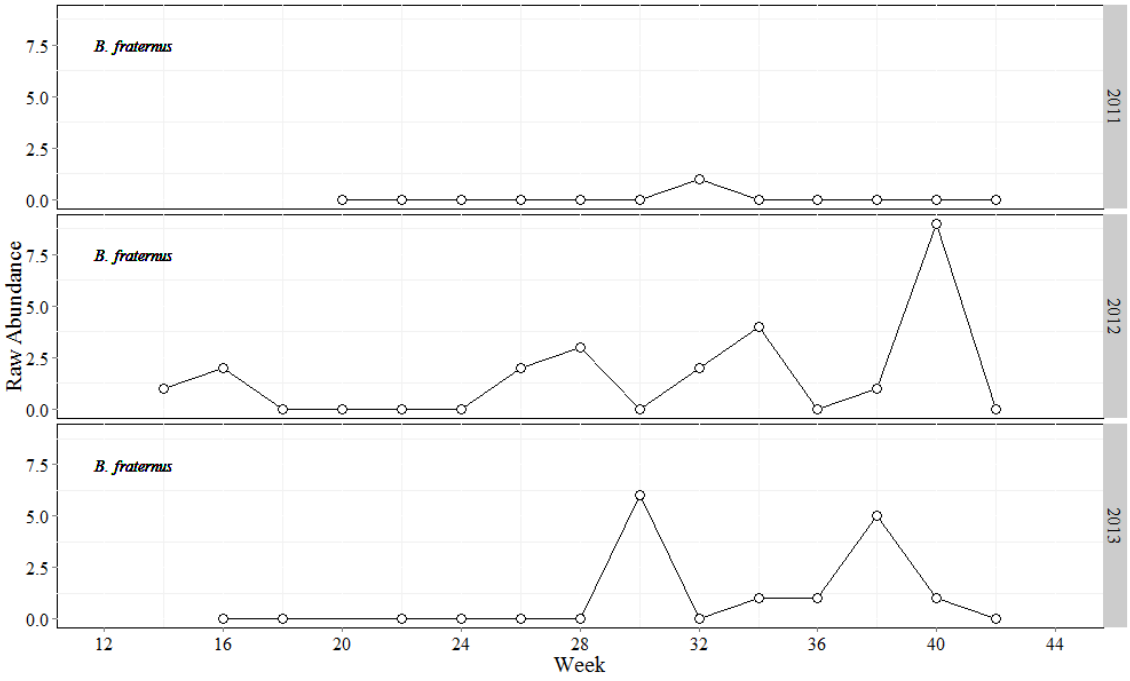

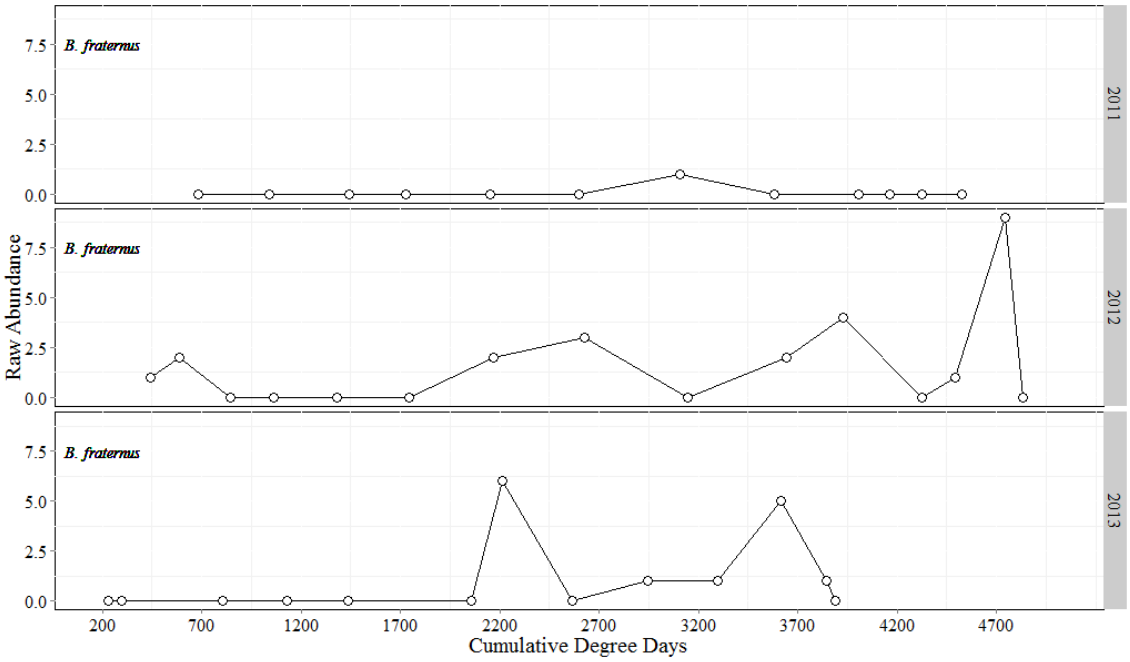

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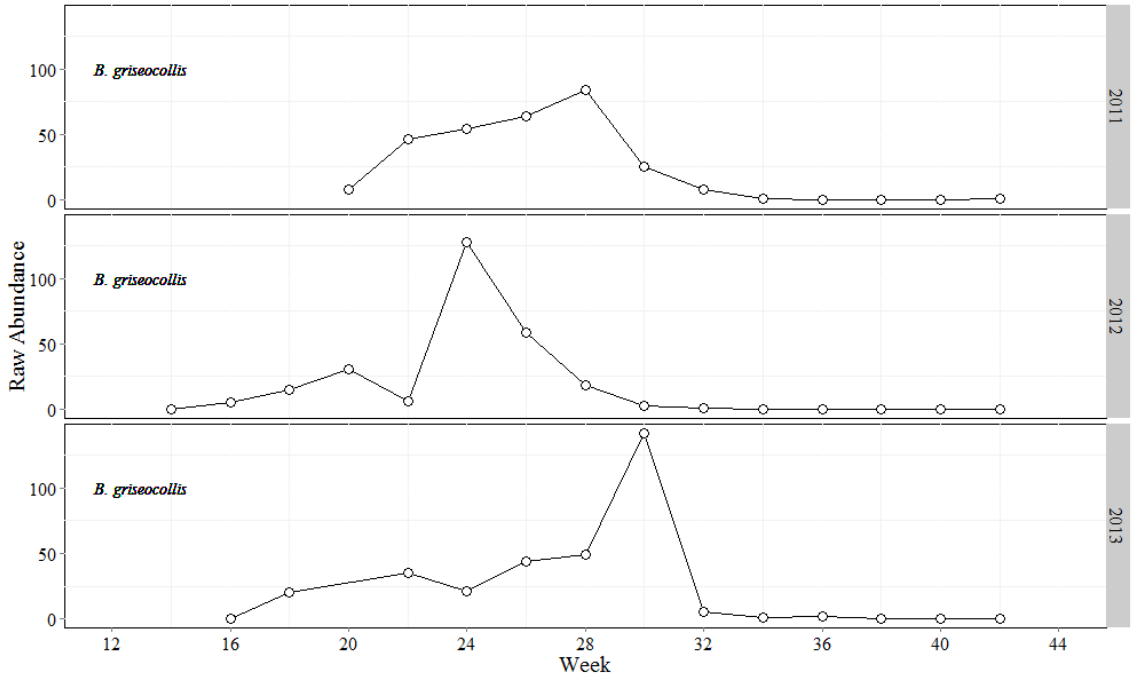

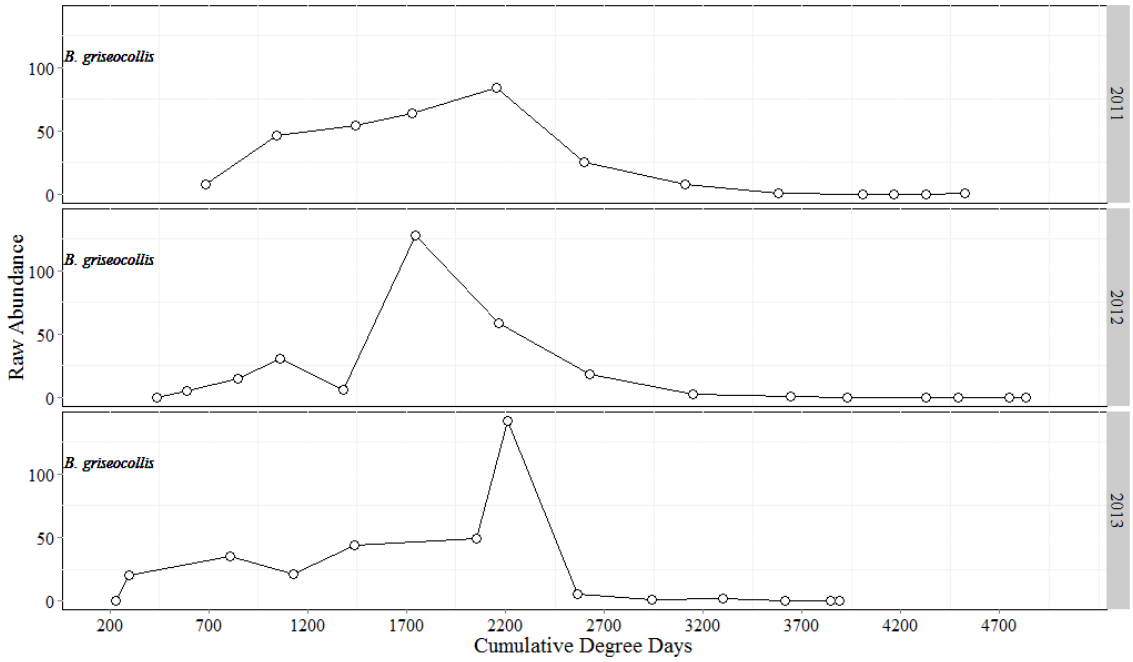

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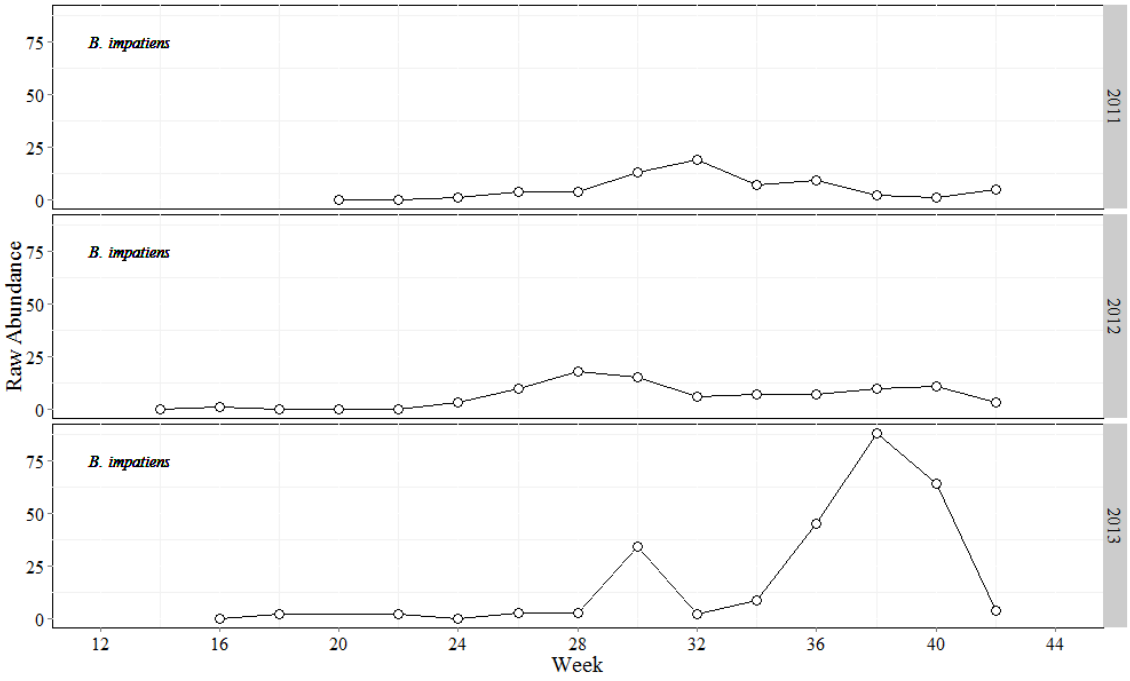

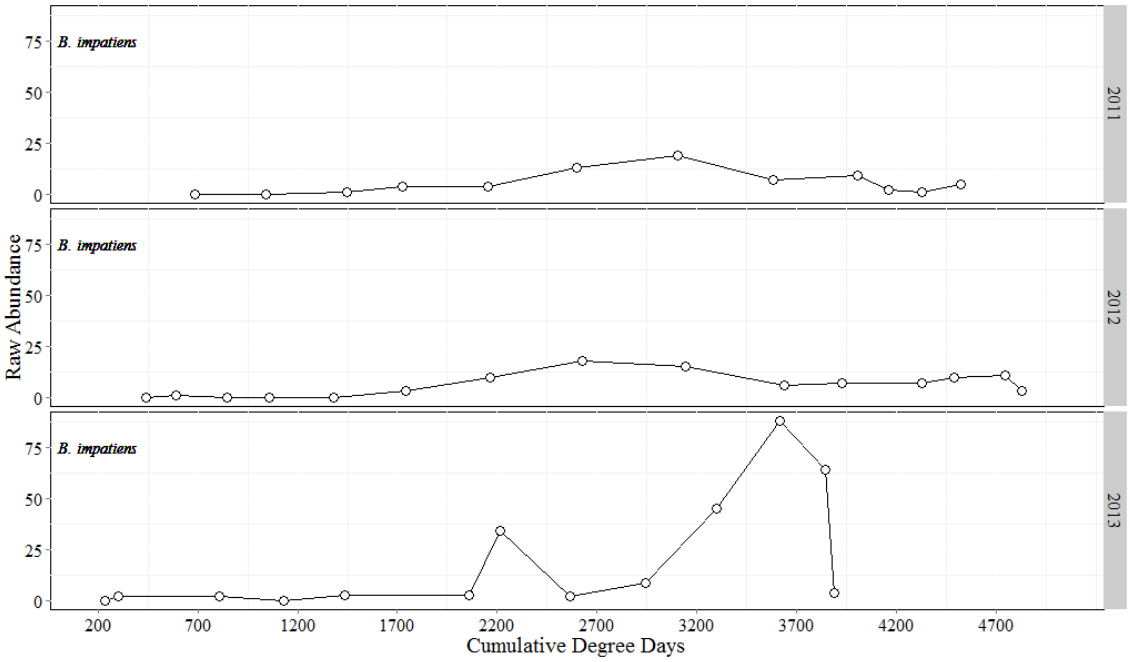

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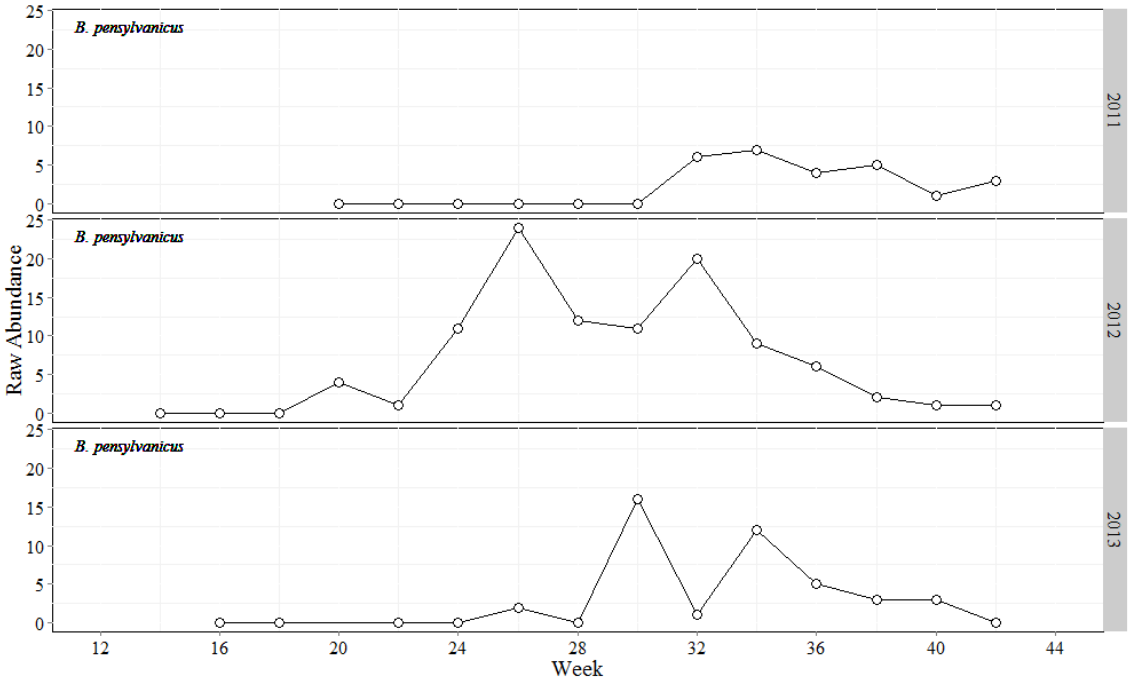

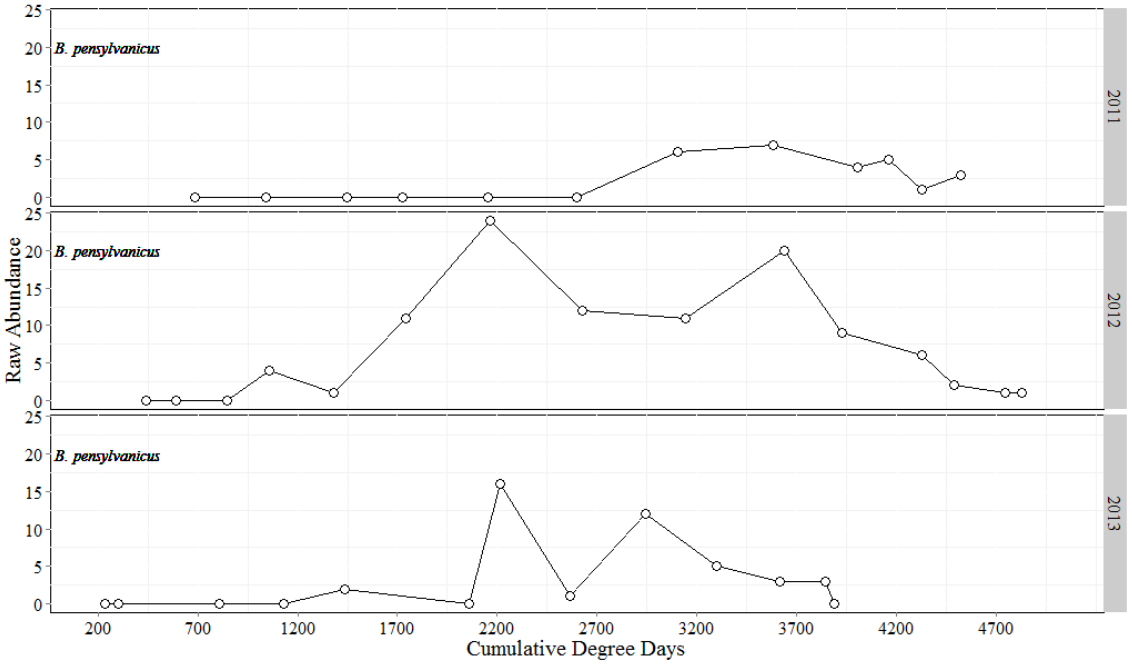

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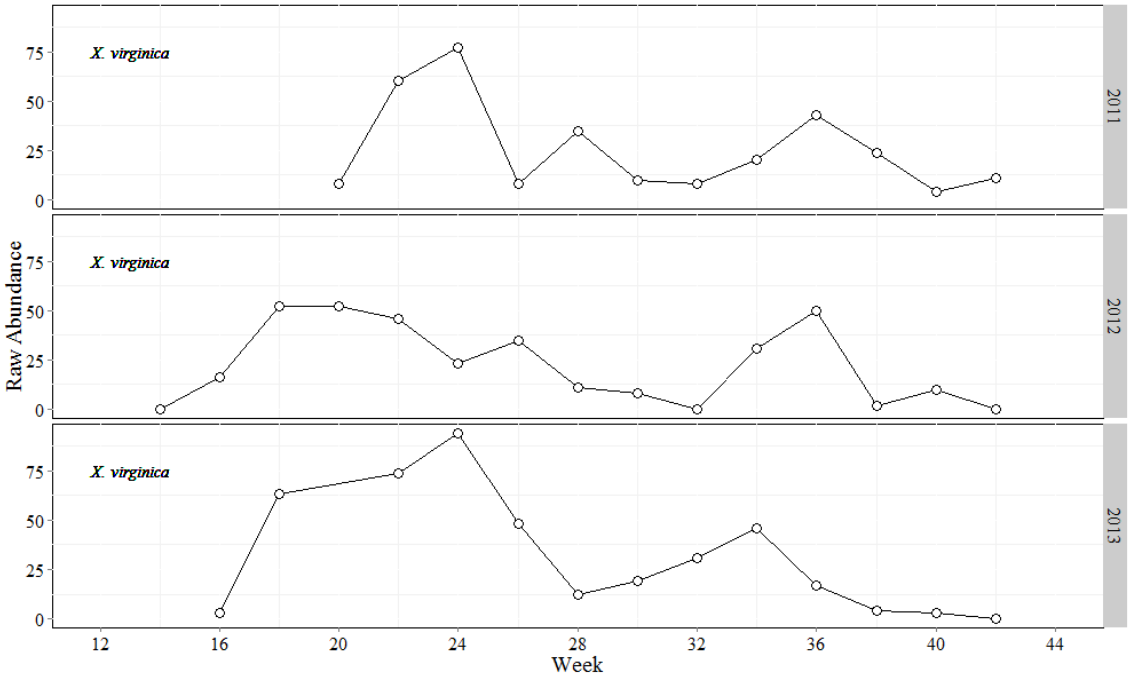

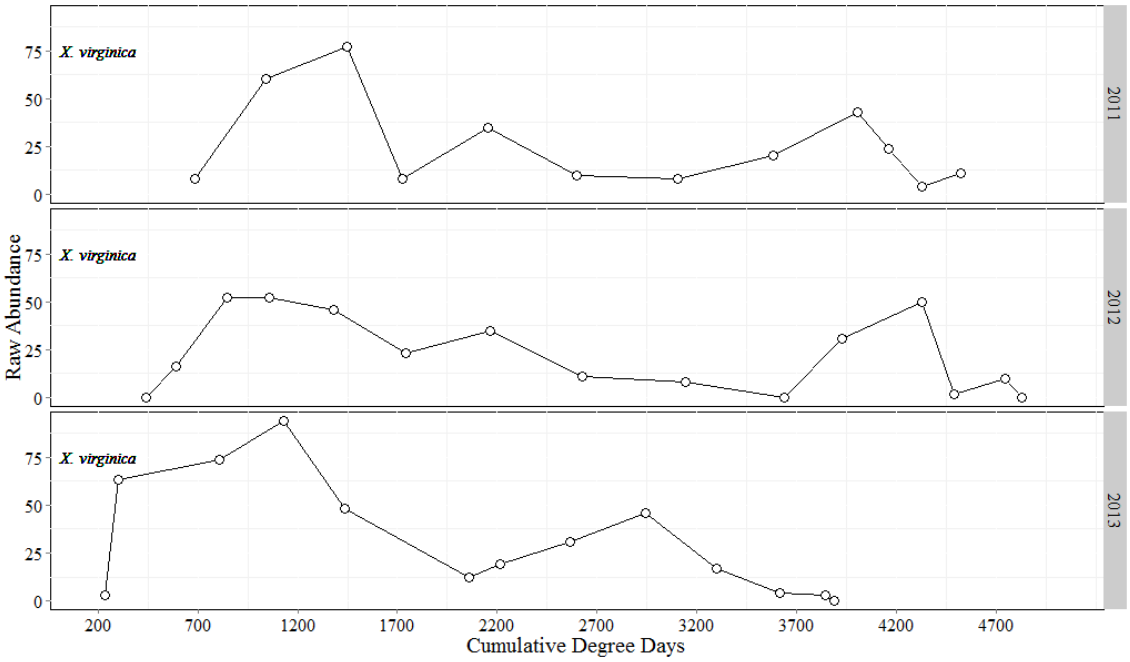

Appendix V.H.5. Factors tested in GLM model selection.

Dependent Variable	Independent Variable	*F*-stat	*df*-deviance	*df*-residual	*p*-value
Diversity Models					
All Bee Diversity (*PIE*)					
Site-Level	Area (ha)	1.33	1	11	0.29
	Plant genera	0	1	10	1
	% native plant species	2.92	1	9	0.13
	% development at site	2.7	1	8	0.14
	% wooded area at site	0.1	1	7	0.76
Local-Level	% development at 250m	0.01	1	11	0.94
	% wooded area at 250m	0.52	1	10	0.49
Landscape-Level	% development at 2000m	3.05	1	11	0.11
	% wooded area at 2000m	2.91	1	10	0.12
Bombus Diversity (*PIE*)					
	Area (ha)	0.11	1	11	0.75
	Plant genera	4.22	1	10	0.079
	% native plant species	0.13	1	9	0.73
	% development at site	1.41	1	8	0.27
	% wooded area at site	6.16	1	7	0.042
Local-Level	% development at 250m	0.3	1	11	0.6
	% wooded area at 250m	0.79	1	10	0.39
Landscape-Level	% development at 2000m	1.64	1	11	0.23
	% wooded area at 2000m	0.37	1	10	0.56
Abundance Models					
Total bees					
Site-Level	Area (ha)	4.04	1	11	0.084
	Plant genera	**45.6**	**1**	**10**	**<0.001**
	% native plant species	4.02	1	9	0.08
	% development at site	0.3	1	8	0.6
	% wooded area at site	0.29	1	7	0.61
Local-Level	% development at 250m	0.01	1	10	0.94
	% wooded area at 250m	0.52	1	11	0.49
Landscape-Level	% development at 2000m	0.17	1	10	0.69
	% wooded area at 2000m	0.01	1	11	0.91

Appendix V.H.5. (Cont.)

Dependent Variable	Independent Variable	*F*-stat	*df*-deviance	*df*-residual	*p*-value
Diversity Models (Cont.)					
Total Bombus (Cont.)					
Site-Level	Area (ha)	4.33	1	11	0.076
	Plant genera	**30.3**	**1**	**10**	**<0.001**
	% native plant species	1.95	1	9	0.21
	% development at site	5.57	1	8	0.05
	% wooded area at site	0.31	1	7	0.6
Local-Level	% development at 250m	0.75	1	10	0.41
	% wooded area at 250m	1.48	1	11	0.25
Landscape-Level	% development at 2000m	0.7	1	10	0.42
	% wooded area at 2000m	0.33	1	11	0.58
B. auricomus					
Site-Level	Area (ha)	1.08	1	11	0.33
	Plant genera	**51.5**	**1**	**10**	**<0.001**
	% native plant species	11	1	9	0.013
	% development at site	2.49	1	8	0.16
	% wooded area at site	0.25	1	7	0.63
Local-Level	% development at 250m	0.66	1	10	0.44
	% wooded area at 250m	2.23	1	11	0.17
Landscape-Level	% development at 2000m	0.94	1	10	0.36
	% wooded area at 2000m	0.91	1	11	0.36
B. bimaculatus					
Site-Level	Area (ha)	2.81	1	11	0.14
	Plant genera	0.28	1	10	0.61
	% native plant species	0.42	1	9	0.54
	% development at site	6.06	1	8	0.043
	% wooded area at site	5.84	1	7	0.046
Local-Level	% development at 250m	1.09	1	10	0.32
	% wooded area at 250m	3.4	1	11	0.095
Landscape-Level	% development at 2000m	1.62	1	10	0.23
	% wooded area at 2000m	5.23	1	11	0.045

Appendix V.H.5. (Cont.)

Dependent Variable	Independent Variable	*F*-stat	*df*-deviance	*df*-residual	*p*-value
Diversity Models (Cont.)					
B. fraternus					
Site-Level	**Area (ha)**	**82.4**	**1**	**11**	**< 0.001**
	Plant genera	**134**	**1**	**10**	**< 0.001**
	% native plant species	4.26	1	9	0.078
	% development at site	**14.4**	**1**	**8**	**< 0.01**
	% wooded area at site	3.44	1	7	0.11
Local-Level	% development at 250m	0.09	1	10	0.77
	% wooded area at 250m	3.35	1	11	0.097
Landscape-Level	% development at 2000m	0.17	1	10	0.68
	% wooded area at 2000m	3.15	1	11	0.11
B. griseocollis					
Site-Level	Area (ha)	3.33	1	11	0.11
	Plant genera	7.31	1	10	0.03
	% native plant species	1.02	1	9	0.35
	% development at site	2.18	1	8	0.18
	% wooded area at site	0.21	1	7	0.66
Local-Level	% development at 250m	0.22	1	10	0.65
	% wooded area at 250m	2.54	1	11	0.14
Landscape-Level	% development at 2000m	0.03	1	10	0.87
	% wooded area at 2000m	0.58	1	11	0.46
B. impatiens					
Site-Level	Area (ha)	1.22	1	11	0.31
	Plant genera	**23.3**	**1**	**10**	**<0.01**
	% native plant species	0.09	1	9	0.77
	% development at site	3.76	1	8	0.094
	% wooded area at site	<0.001	1	7	0.98
Local-Level	% development at 250m	1.28	1	10	0.28
	% wooded area at 250m	1.14	1	11	0.31
Landscape-Level	% development at 2000m	1.35	1	10	0.27
	% wooded area at 2000m	0.37	1	11	0.55
B. pensylvanicus					
Site-Level	Area (ha)	<0.001	1	11	1
	Plant genera	**22.6**	**1**	**10**	**<0.01**
	% native plant species	1.3	1	9	0.29
	% development at site	7.99	1	8	0.026
	% wooded area at site	11.9	1	7	0.011

Appendix V.H.5. (Cont.)

Dependent Variable	Independent Variable	*F*-stat	*df*-deviance	*df*-residual	*p*-value
Diversity Models (Cont.)					
B. pensylvanicus(Cont.)					
Local-Level	% development at 250m	<0.001	1	10	0.98
	% wooded area at 250m	0.16	1	11	0.7
Landscape-Level	% development at 2000m	0.56	1	10	0.47
	% wooded area at 2000m	0.06	1	11	0.81
A. mellifera					
Site-Level	Area (ha)	<0.001	1	11	0.98
	Plant genera	10.9	1	10	0.013
	% native plant species	7.01	1	9	0.033
	% development at site	0.32	1	8	0.59
	% wooded area at site	0.03	1	7	0.87
Local-Level	% development at 250m	0.33	1	10	0.58
	% wooded area at 250m	0	1	11	0.97
Landscape-Level	% development at 2000m	2.86	1	10	0.12
	% wooded area at 2000m	1.29	1	11	0.28
X. virginica					
Site-Level	Area (ha)	6.37	1	11	0.04
	Plant genera	1.27	1	10	0.3
	% native plant species	1.37	1	9	0.28
	% development at site	1.85	1	8	0.22
	% wooded area at site	0	1	7	0.97
Local-Level	% development at 250m	0.01	1	10	0.94
	% wooded area at 250m	2.39	1	11	0.17
Landscape-Level	% development at 2000m	1.87	1	10	0.2
	% wooded area at 2000m	**8.46**	**1**	**11**	**0.016**

Factors retained in models are shown in bold. Alpha levels for significance adjusted with Bonferroni corrections by dividing 0.05 by the number of factors tested in each model.

VI. REVISITING THE SUBSPECIES OF *XYLOCOPA VIRGINICA* (LINNAEUS, 1771) (HYMENOPTERA: APIDAE)

A. ABSTRACT

Three *Xylocopa* (*Xylocopoides*) *virginica* (Linnaeus, 1771) subspecies have been described: the nominal and ubiquitous *X. v. virginica*, the Texas-Oklahoma restricted *X. v. texana* Cresson, 1872 and the south-Floridian *X. v. krombeini* Hurd, 1961. Setal variation thought to be restricted to *X. v. krombeini* is widespread throughout the range of *X. v. virginica*, and the two subspecies are not diagnosably distinct from one another. On the other hand, both males and females of *X. v. texana* can be readily diagnosed, and their distinctive characters are somewhat geographically constrained west of about 95°W latitude, although the distributions of the nominal subspecies and *X. v. texana* overlap where these subspecies meet. Diagnosis of *X. v. texana* females is rather straightforward as they have a large frontal carina, square-shaped tibial scales and an iridescent sheen on the dorsum of the metasoma. Male *X. v. texana* can be distinguished by the presence of dark pigmentation in the paraocular area, and often have pale bands apico-dorsally on T4 and an iridescent metasoma. This work proposes the synonymy of *X. v. krombeini* with *X. v. virginica,* and provides an updated dichotomous key and distribution maps for *X. v. texana* and *X. v. virginica.*

B. INTRODUCTION

Populations within a species that are morphologically, ecologically or genetically distinct and geographically cohesive are often granted the status of subspecies, a status formally codified with a trinomial. Ideally, a subspecies should classify populations in such a way as to allow further, in-depth investigations into the evolutionary histories, adaptiveness and other such characteristics that are relevant to many biological, and not just taxonomic, fields (Barrowclough

1982). However, incorrect classifications can just as readily lead to false inferences and misdirect both scientific and conservation efforts (Zink 2004). A population that is elevated to the status of a subspecies by being given a formal trinomial is expected to fulfill certain criteria, but diagnosability can be considered one of the most important (Braby, *et al.* 2012).

Subspecies are common within the *Xylocopa* of North America. For example, *X.* (*Xylocopoides*) *californica* Cresson, 1864 includes three recognized subspecies, and *X.* (*Notoxylocopa*) *tabaniformis* Smith, 1854 includes ten, three of which are present in the American West (Hurd 1978). Some subspecies of *X. tabaniformis* differ in their diurnal activity period, with some subspecies active mid-day and some crepuscular (Janzen 1964; O'Brien and Hurd 1965). Yet, for the taxonomist working with preserved specimens, there are no morphological characters that can be used to diagnose these subspecies of *X. tabaniformis*, and subspecies designations are to be applied on the basis of geographic origin alone (O'Brien and Hurd 1965).

There are three recognized subspecies of *X.* (*Xylocopoides*) *virginica* (Linnaeus, 1771): the nominal and ubiquitous *X. v. virginica*, the Texas-Oklahoma restricted *X. v. texana* Cresson, 1872 and the south-Floridian *X. v. krombeini* Hurd, 1961 (Fig. VI.B.1) (Hurd and Moure 1963). The three subspecies of *X. virginica* were described based on external morphology and geographic distribution alone (characters are summarized in Table VI.B.1) and are said to occur allopatrically (Hurd and Moure 1963) (Fig. VI.B.1). The existence of three subspecies with allopatric distributions suggests that there are multiple, discrete lineages of *X. virginica*. A critical examination of the morphology and distributions of these subspecies was undertaken prior to a molecular phylogeographic study of this species throughout its range.

Xylocopa texana was considered an independent species until 1955 when it was demoted to subspecific status based on morphological characters and geographic distribution (Hurd 1955). It is distributed west of the range of *X. v. virginica* in Texas, Oklahoma and a small part of southern Kansas. In his description, Cresson (1872) stated that *X. texana* is most easily distinguished from *X. v. virginica* by the blue-green iridescence of the metasoma of both sexes. This character is subtle (Fig. VI.B.2. *cf.* A and B) and can be difficult to see in many preserved specimens, however. Ackerman (1916) suggested that the presence of light-colored pile on posterior segments of *X. texana* (males: dorso-laterally on metasomal tergites T4 and T5; females: laterally on T5 and T6) was a more dependable character. Metasomal pile characters, along with metasomal color, were also used by Hurd (1955; 1961) to distinguish *X. v. texana* in keys, but Ackerman (1916) preferred to use the presence of pale setae between the antennal sockets and on the cheeks to identify *X. v. texana*. The paraocular area is typically fully or mostly black on male specimens of *X. v. texana*, but in *X. v. virginica*, this region is pale yellow like the clypeus of both subspecies. This character is apparently variable in male *X. v. texana*, however, and Ackerman (1916) speculated that this character might not be sufficient for distinguishing males of the two subspecies. Although Ackerman (1916) stated that he felt confident that the genitalia differed between *X. virginica* and *X. texana* males, his illustrations are unclear, and he offered no descriptions. Hurd (1955) disagreed, stating that their genitalia are "virtually identical".

Although all *X. v. virginica* females exhibit a large, frontal carina (*sensu* Ackerman 1916), (also referred to as an interantennal crest or tubercle (Hurd 1955) or frontal keel (Michener 1954)) between the antennal sockets, this is quite large in females of *X. v. texana* (Fig. VI.B.2 *cf.* F and G) and, along with metasomal color, is used to distinguish *X. v. texana* females

in keys (Ackerman 1916; Hurd 1961). Diagnosing females with this character might also be difficult as the size of the carina is claimed to be larger in *X. v. virginica* specimens from Texas than in specimens from further east (Ackerman 1916). This suggests clinal variation, rather than a character associated with a discrete geographic population. Whether or not this projection serves a function externally is unknown, but muscles that control the labrum are attached to this integument on its hollow interior (personal observation).

Ackerman (1916) also noted that the tibial scale on the hind leg of females differs between *X. v. virginica* and *X. v. texana.* Although the tibial scales are uniquely cupped (foveate) within the subgenus *Xylocopoides* (Hurd 1961), this character was not included in any keys or descriptions of *X. virginica* subspecies by Hurd (1955; 1961). The tibial scale is described as rather square in *X. v. texana*, with the apical teeth of equal length, whereas in *X. v. virginica*, the scale is elongated, and the posterior tooth is shorter and more rounded than the anterior one (Ackerman 1916).

In 1961, Hurd designated *X. v. krombeini* as a subspecies of *X. virginica* restricted to the southern portion of Florida based on material collected from Lake Placid in Highlands, Co. Florida (Hurd 1961). This subspecies shares characteristics of both *X. v. virginica* and *X. v. texana*. According to Hurd (1961), *X. v. krombeini* males are distinguished from *X. v. virginica* by the presence of white pubescence laterally on the apices of T4-T6 and a "narrow" ring of black pubescence surrounding the setae-free area on the dorsum of the thorax (Hurd 1961). Unlike in *X. v. texana* males, in *X. v. krombeini* the white pubescence on T4 does not extend dorsally. Like males, females of *X. v. krombeini* exhibit pale pubescence laterally on T5, and often on T4 and T6, but they are distinguished from *X. v. texana* by their less protuberant frontal carina (Hurd 1961). Within his key to females, Hurd (1961) designated the pale setae on T4-T6

(as IV-VII, but presumably, since only six terga are visible on females, the inclusion of T7 as a female character was a typographical error) as diagnostic for *X. v. krombeini*, claiming the apices of T4-T6 to be "entirely dark pubescent" in *X. v. virginica.* In his description of *X. v. krombeini*, Hurd (1961) described an aberrant female specimen from Paradise Key, Florida with white pubescence apically on the metasomal sterna as well as on the outer surface of the hind tibia and tarsi. Although he suggested that this might represent another subspecies of *X. v. virginica* restricted to the Florida Keys, it is also possible that it is indication of how variable setal characteristics can be in *X. virginica*.

Literature references to *X. virginica* subspecies are rare. Older references indicate that the morphological variation in *X. virginica* has been the source of some confusion. Ashmead (1894) casually referred to *X. v. texana* (as *X. texana*) being "common at Jacksonville, Florida", a mistaken point repeated even after Hurd's (1961) revision of *X. virginica* subspecies (Balduf 1962). I could find only two published references to *X. v. krombeini*. One is an extension publication from Florida (Grissell 1975). The other included *X. v. krombeini* in a list of 89 Hymenopteran taxa surveyed for the size of their genomes, with no identification reference (Frankie and Vinson 1977). References to *X. v. texana* are more numerous, but lacking references for identification methods, subspecific identifications seem to be based solely upon geographic location without reference to morphology (*e.g*. Frankie and Vinson 1977; Williams, *et al.* 1983; Barthell and Baird 2004; Barthell, *et al.* 2006). It is clear that contemporary researchers are accepting the subspecies of *X. virginica*, but it is unclear how critically they are examining this status.

Objectives

This work seeks to resolve two major questions related to Hurd's (1961) taxonomic hypotheses: 1) are the subspecies of *X. virginica* morphologically diagnosable? and 2) are their distributions geographically discrete?. The three *X. virginica* subspecies have been described as having discrete morphologies and allopatric distributions (Hurd 1961), but these properties have not been examined in depth. Additionally, existing keys lead to incorrect classifications and are largely dependent upon the geographic location of a specimen, rather than its morphology. I hypothesize that the subspecies of *X. virginica* show overlap in their morphological distinctions and distributions, contrary to their status as described by Hurd & Moure (1963). Instead, I expect that the variation seen in *X. virginica* is ascribable to population-level polymorphisms, rather than subspecies status.

C. Materials and Methods

Specimen Acquisition and Identification

Xylocopa specimens were obtained from the University of Arkansas Arthropod Museum (UAAM), Florida State Collection of Arthropods (FSCA) and Texas A&M University Insect Collection (TAMU). Locality data were collected for all specimens of *X. virginica* contained in these collections. Specimens collected by the author or donated to the author were included in the analysis and have been deposited in UAAM. Each specimen was identified to species using the morphological key of Hurd and Moure (1963). Because there were specimens with mixed morphology, this key was unable to resolve *X. virginica* subspecies. Instead, character states were scored for each *X. virginica* specimen using the characters listed in Table VI.C.1, then subsequently analyzed as described below. Most scored characters were discrete; only the female carina size was recorded as a ratio. Tibial scale drawings were conducted at a magnification of

16x and executed at scale of 1:60. The holotype of *X. v. krombeini* was examined from photographs of the male specimen deposited at the United States National Museum of Natural History (#00534336, available at http://collections.nmnh.si.edu, accessed 12 Jul, 2014).

Character States

A summary of the character rubric is provided in Table VI.C.1. As all *X. virginica* exhibit a fair amount of iridescence on T1, the color of the metasoma was classified based on segments T2–T6 (females) or T2–T7 (males). This character was scored as "black" if there was no visible iridescent sheen on these terga and as "iridescent" if a green or blue-green iridescence was observed (Fig. VI.B.2.A–B). The extent of black setae on the dorsum of the thorax of males was estimated by drawing an imaginary line between the bases of the tegulae and comparing the width of the black patch along that line to the extent of yellow setae along that line between the edge of a tegula and the start of the black patch of setae. Specimens with the width of the black patch exceeding the extent of yellow setae on one side were scored as having a "large black spot" (Fig. VI.B.2.E, *cf.* D). The size of the patch of pale setae on the ventrolateral aspect of T4-T6 was classified as "black" (no pale setae present), "few" (<20 pale setae) or "large" (>20 pale setae). The size of the carina in females was estimated as a ratio of the height of the carina to the distance from the edge of the antennal base to the midline of the carina. For some analyses, this ratio was classified as a binary variable as either <1 (Fig. VI.B.2.F) or ≥1 (Fig. VI.B.2.G). The shape of the tibial scale was classified as either "elongate" in form, with the length exceeding the width (Fig. VI.C.1.A), or "square" with length and width approximately equal (Fig. VI.C.1.B) when viewed in a flat plane.

Geo-referencing Sampling Localities

Many samples obtained from museums and other collectors were not associated with latitude-longitude coordinates, and the precision of the locality data included with each specimen varied. Some specimens were labeled with detailed information (*e.g.* street address, road intersection, distance from landmarks), but most labels listed only the city or county in which the specimen was taken. County was the most consistent locality level in the museum specimens, thus I chose this as the level at which locality was recorded for each specimen. Because this study covers such a large geographic area, deviances from actual sample locations should not influence the conclusions drawn. All geo-referenced samples were added to ARCGIS v10.0 (ESRI, Redwood, CA) for spatial analysis.

Decision Criterion

In addition to considerations of geographic discreteness, the 75% rule (Amadon 1949) was applied as a general decision threshold in population-level analyses. Because all characters except the size of the carina ratio were discrete and categorical, diagnosability was assessed by comparing simple frequencies to a 75% threshold (Patten and Unitt 2002). For example, the determination of whether a character in a polymorphic population should be classified as fixed or ambiguous was decided by the frequency of the state in the population. If a state was found in greater than 75% of the individuals sampled in the population, the population itself was scored as characterized by that character state. If less than 75% of individuals exhibited a single character state, the state was scored as ambiguous. All population-level analyses were conducted on populations with five or more individuals, and males and females were analyzed separately.

Analyses

The discreteness of ranges was examined by tallying the percent of individuals exhibiting expected character states within each subspecies' range. In order to determine congruence between morphological states and geographic location, a cladistics, tree-based approach using population aggregation analysis methods (Davis and Nixon 1992) was adopted (Wiens and Penkrot 2002). A population profile was created for each population with five or more sampled specimens, with characters scored as fixed (present in > 75% of individuals) for a state or ambiguous (present in ≤ 25% of individuals). Ambiguity was treated as an additional character state. An unrooted tree was then constructed for male and female character matrices separately using populations as the terminal taxa. Trees were constructed using Maximum Parsimony, with a heuristic parsimony ratchet search (Nixon 1999) using subtree pruning and regrafting to determine the optimal topologies in R (R Core Team 2014) with the packages *ape*, *phangorn* and *phytools* (Paradis, *et al.* 2004; Schliep 2011; Revell 2012). A Majority-Rule-Consensus tree was constructed from all trees of optimal length, and estimates of node support were estimated with 1,000 non-parametric bootstrap samples.

D. RESULTS

A total of 899 *X. virginica* specimens were examined and scored (n_{female}=477, n_{male}=422). This material covered 179 counties in 22 states throughout the range of the species in the eastern United States (Fig. VI.B.1). There were 39 specimens examined from the expected range of *X. v. krombeini*, 156 from the expected range of *X. v. texana*, 678 from the expected range of *X. v. virginica*, 21 examined from areas of overlap between subspecies ranges and five without locality data. The geographic distributions of each variable character state are shown in Figure VI.D.1 A–J for males and Figure VI.D.2 A–H for females. Characters on the dorsum of the

thorax were frequently worn or obscured (*e.g.* Fig. VI.B.2.C.). This was the case for 21% of both male and female specimens (n_{male}=88, n_{female}=100).

The carina ratio was larger in females from the *X. v. texana* range (0.90±0.30 SD) than in females from either of the other two ranges (*X. v. krombeini:* 0.52±0.07 SD; *X. v. virginica:* 0.53±0.10 SD). There is little indication of an east-west cline in this ratio, but no specimen west of -98.5° longitude exhibited a carina-to-antennal-base ratio of less than one, and no specimen east of -95.9° exhibited a ratio of one or more (Fig. VI.D.3). The mean ratio among classified specimens showed a more pronounced trend, with *X. v. texana* specimens having an average ratio larger (1.14±0.23 SD, red circle in Fig.VI.D.3) than that of the remaining specimens, whether these were grouped (0.54±0.12, black circle in Fig.VI.D.3) or separate (*X. v. krombeini:* 0.50 ± 0.04 SD; *X. v. virginica:* 0.55±0.12, gold and black squares, respectively, Fig. VI.D.3). The hind tibial scale morphology of females (Fig. VI.C.1) showed a similar geographic discreteness (Fig. VI.D.2.G–H). A single specimen exhibiting the square-shape-with-equal-teeth morphology (Fig. VI.C.1.B) was found east of -95.9° longitude. This Washington County, Arkansas female exhibited no other characters deviating from the expected morphology of *X. v. virginica.* Among all females, the overall shape of the scale was well-correlated with the relative tooth length (Pearson's ϕ^2=0.95, p<0.05), so the latter character was excluded from subsequent analyses. One female had pale setae on the hind tibia (Sumter County, Florida); this rare character was also excluded from analyses. This specimen matched Hurd's (1961) description of an aberrant *X. v. krombeini* specimen from Paradise Key, Florida. No females exhibited apico-dorsal, pale bands on T4 or T5, so these characters were likewise excluded. Most females exhibited at least some black setae at the dorsum of the thorax (72%), regardless of geographic location (Fig. VI.D.2.B). The size of pale patches on T4-T6 did not seem congruent with

expected ranges (Figs VI.D.1.F,H,J and 2.D–F), so small and large patches were combined for analysis (Table VI.C.1). Contrary to expectations (Hurd 1961), only 12% of females in the *X. v. krombeini* range had pale patches on T6 (Table VI.D.1). The presence of a patch of pale setae on the ventrolateral aspect of T5 was common among females in both the *X. v. texana* (43%) and *X. v. krombeini* (83%) ranges, yet rarer in the range of the nominal subspecies (25%). Using Amadon's (1949) 75% rule, this character is close, but not sufficiently exclusive for separation of *X. v. virginica* and *X. v. krombeini* females.

The metasomal iridescence (Fig. VI.B.2.A) favored by both Ackerman (1916) and Hurd (1961) as diagnostic for both males and females of *X. v. texana* was less reliable than expected. Only 60% of males and 40% of females in the range of *X. v. texana* exhibited this character (Table VI.D.1). However, specimens with an iridescent metasoma were more common in the range of *X. v. texana* than elsewhere, and this region contained 68% of specimens exhibiting this character. The presence of pale setae between the antennal bases and on the cheeks was not restricted to specimens in the range of *X. v. texana*, contrary to Ackerman's (1916) claim (Fig. VI.D.1.B–C). The paraocular area of male specimens mirrored the geographic distribution of female carina ratios and tibial scale morphology (Fig. VI.D.1.A). The degree of dark coloration in this area, whether mixed with pale (Fig. VI.D.4.B) or solid black (Fig. VI.D.4.C), did not seem correlated with geography, so these characters were combined, and paraocular area coloration was analyzed as a binary variable (Table VI.C.1). West of -95° longitude, 20% of males (n=16) exhibited fully pale paraocular areas (Fig. VI.D.4.A); east of this longitude, less than 1% (n=3) exhibited any dark pigmentation in the paraocular area. The paraocular area of *X. v. texana* males almost invariably included black pigmentation (50% black, 49% mixed), and this single character seems to be reliably diagnostic for the subspecies. A single specimen on the edge of the

expected *X. v. texana* range in Latimer County, Oklahoma lacked dark paraocular pigmentation, yet exhibited a dorsal fringe of pale setae on T4 and an iridescent metasoma and was classified as *X. v. texana*. Males from the *X. v. krombeini* range were more likely to have patches of pale setae ventrolaterally on T5 and T6 (93% in both cases) than males in the *X. v. virginica* range (15% and 7%, respectively) or the *X. v. texana* range (34% and 25%, respectively) (Table VI.D.1). Although the presence of a large patch of black setae on the dorsum of the thorax (Fig. VI.B.2.E) was common in males from Florida (n=30), this character was also common in Louisiana (n=23), Maryland (n=11) and New Jersey (n=6) (Fig. VI.D.1.D). Males exhibiting both a large black spot on the thorax and pale patches on T5 were found in Texas (n=1), Louisiana (n=6), Maryland (n=1) and in Florida north of the expected *X. v. krombeini* range (n=5). Although this combination of characters was present in far less than 25% of the *X. v. virginica* sample, it is widely distributed outside of the expected range, with 50% of samples exhibiting this morphology occurring outside of the expected range of *X. v. krombeini*.

Cladistic analyses

Fifteen populations of males were sampled well enough to include in cladistic analyses (population profiles, Appendix VI.I.1). Maximum Parsimony analysis yielded 26 most-parsimonious trees of length=25, with a consistency index of 0.61 and a retention index of 0.63 (Fig. VI.D.5). All taxa ultimately formed an unresolved polytomy, with only five resolved clades with bootstrap values above 50%. Populations did show some congruence between morphology and geography, however. The Texas Counties Kerr and Uvalde formed a single, well-supported clade (Fig. VI.D.5, clade 2), sister to the Brazos County, Texas population (Fig. VI.D.5, clade 1). All three populations are in the range of *X. v. texana*, and together are synapomorphic for having the paraocular area black (Fig. VI.D.5, clade 1). Members of the Kerr and Uvalde populations

are further characterized by the unambiguous presence of an iridescent metasoma, an interrupted fringe of pale setae on the apex of T4, accompanied by lateral patches of pale setae on T4 and T5. Brazos County is in the eastern extreme of the recorded range of *X v. texana,* and both *X. v. texana* (n=35) and *X. v. virginica* (n=43) were common there. The single population representing the range of *X. v. krombeini* (Highlands County, Florida) was undifferentiated from the greater polytomy, (Fig. VI.D.5). Although Highlands County, Florida was the only population to unambiguously exhibit the full suite of *X. v. krombeini* characters (*i.e.* a large patch of black setae on the dorsum of the thorax and pale patches on T5 and T6), the presence of these states among other populations, either in full or in part, prevented the separation of this group (Appendix VI.I.1). No population unambiguously presented the full suite of expected *X. v. virginica* characters (as shown in Table VI.B.1).

Twenty-four populations of females were available for cladistic analyses (population profiles, Appendix VI.I.2). For these data, Maximum Parsimony analysis yielded 2 most-parsimonious trees of length=19, with a consistency index of 0.65 and a retention index of 0.80 (Fig. VI.D.6). As in the male tree, phylogenetic resolution was low, with few nodes supported by bootstrap values above 50%. Five populations from the range of *X. v. texana* were grouped together (Fig. VI.D.6, clade 1), although only the clade containing the Texas Counties Kerr and Bosque was well supported. The Bosque population was the only population that exhibited unambiguous *X. v. texana* morphology, but all populations in this clade were united by a synapomorphic square tibial scale. All populations in this clade were also exclusive in that they had members with large carinas, but this character was ambiguous in the Cleveland County, Oklahoma population (n_{large}=5, n_{small}=3). A second clade, eight nodes removed from the majority of *X. v. texana* populations (Fig. VI.D.6, clade 2), contained the other two Texas populations:

Brazos County, which is in the range of *X. v. texana,* and Montgomery County, which is in the overlapping area containing both *X. v. virginica* and *X. v. texana.* All Montgomery County females were morphologically diagnosed as *X. v. virginica,* but 10% of females in the Brazos County population were diagnosed as *X. v. texana* (n=4 of 41 total). This Texas clade is the terminus of the clade grouping all populations with mixed black and yellow setae on the dorsum of the thorax, but otherwise unambiguously matching expected *X. v. virginica* morphology across all scored characters for >75% of the examined specimens (Fig. VI.D.6, clade 2). Atchison County, Kansas was the only population whose members perfectly matched expected morphology of *X. v. virginica* in that all members had pure yellow setae on the dorsum of the thorax. Populations in the clade marked number 3 in Fig. VI.D.6 unambiguously matched expected *X. v. virginica* morphology in all characters, with the exception of the setal color on the dorsum of the thorax. Each of these populations exhibited a combination of members with pure yellow and mixed setae such that this character was scored as an ambiguity in each population. No population exhibited the expected suite of *X. v. krombeini* characters. Sumter County, Florida was the only population examined from the expected range described of *X. v. krombeini,* and this population formed a well-supported clade with Columbia County, Florida, characterized by having mixed setae on the dorsum of the thorax, ambiguity for pale patches on T4 and consistency in the presence of pale patches on T5 (Fig. VI.D.6, clade 4). The clade containing Jefferson County, Alabama and Alachua and Duval Counties, Florida that was sister to this clade shared ambiguities in the color of setae on the thorax and on the later of T4. The Alachua population also exhibited patches of pale setae on T5, but the other two populations were ambiguous for this character.

E. DISCUSSION

It is not possible to reliably separate specimens of *X. v .virginica* and *X. v. krombeini*. In my opinion, *X. v. krombeini* should be regarded as a junior synonym of *X. v. virginica.* Of the specimens examined, 115 were diagnosed as *X. v. texana* and 779 as *X. v. virginica.* Five specimens were not diagnosable due to the obliteration of important characters and lack of sufficient locality data. The distributions of *X. v. virginica* and *X. v. texana* are not entirely allopatric. The distributions of diagnosed subspecies are shown in Fig. VI.D.7.

Diagnosis of *X. v. texana* females is rather straightforward using a combination of the carina ratio, shape of the tibial scale and the color of the metasoma. Although *X. v. texana* females can typically be adequately diagnosed by a combination of characters unique to this subspecies, the only characters available to separate female specimens of *X. v. krombeini* from *X. v. virginica* are the presence of pale setae on the sides of T5 and T6 (Hurd 1961). Females outside of the expected *X. v. krombeini* range also have pale setae on the sides of T5 quite frequently. Additionally, the presence of pale setae on T6 of females is uncommon, even in the expected range of *X. v. krombeini*. If Hurd's (1961) description was to be taken strictly and without reference to geography, few female specimens would meet the criteria of a *X. v. krombeini* diagnosis (n=10 of the females examined here; only n=3 in the range of *X. v. krombeini*). Relaxing the diagnosis to the presence of pale setae on the sides of T5 but otherwise matching the description of *X. v. virginica* would cause the range of *X. v. krombeini* to overlap substantially with *X. v. virginica.* Specimens with this character but otherwise matching the description of *X. v. virginica*, were found in Alabama, Arkansas, Florida, Kansas, Louisiana, Maryland, Mississippi, Missouri, Oklahoma, Pennsylvania, South Carolina and Texas (Fig. VI.D.2.E). Males of *X. v. krombeini* are also difficult to diagnose, since the single unique

character, the presence of a large spot of black setae on the thorax, is not restricted to the southern portion of Florida (Fig. VI.D.1.D), and there is little regularity in the distribution of pale patches on T5 (Fig. VI.D.1.H) or T6 (Fig. VI.D.1.J).

Diagnosis of *X. v. texana* males is also straightforward and can usually be accomplished by examination of the coloration of the paraocular area, although the presence of pale bands apico-dorsally on T4 and metasomal iridescence are also associated with the subspecies. Integumental color variation is not unknown in *Xylocopa* species. The clypeus of *X. californica* males is typically dark in color, but specimens from throughout its range often exhibit variation that is not typical of populations and not associated with geographic origin or subspecific designation (Hurd 1955). Similarly, unusual clypeal patterns were observed on a small number of male *X. virginica* specimens, yet these were all collected from Brazos County, Texas. Although the patterns varied, each specimen was marked by atypical black areas of the clypeus (Fig. VI.D.3.D–H). Seven specimens with a total of three pattern types were collected on four occasions in 1954 by A. H. Alex (TAMU). An additional two specimens were collected in 1980 by M. C. Klass, and each exhibited a different pattern (Louisiana State Arthropod Museum Collection, LSAM). All nine specimens exhibited the black paraocular area associated with *X. v. texana*, yet other characters varied among individuals. Ackerman (1916) described an aberrant specimen from Chappell (as Chapel) Hill, south of Brazos County, Texas with a clypeal pattern matching one of the M. C. Klass specimens. The significance of pale facial coloration in males is unknown, but its widespread distribution among disparate bee species suggests that it is both ancestral and important in some sexually-selected aspect (Michener 2013). The clypeal and paraocular color variation exhibited within *Xylocopa* both at the subspecies level and below, suggests that this group might be particularly well-suited to studies of this phenomenon.

There seem to be some recurrent patterns in setal variation among species and subspecies of *Xylocopa* that also might warrant further investigation. For example, males of the subspecies *X. californica californica* differ from other *X. californica* subspecies by the presence of a medially-interrupted band of pale setae sub-apically on T4 (Hurd 1955). Males of two of the three subspecies of *X. tabaniformis* occurring in North America also exhibit this character. In *X. t. androleuca* Michener, 1940, this interrupted band is limited to T4 and T5, making it the main character that separates it from *X. t. parkinsoniae* Cockerell, 1917, which has more dramatic bands on T2-T6 (Hurd 1955). Females of *X.* (*Schonnherria*) *micans* Lepeletier, 1841, a species whose distribution overlaps with all three *X. virginica* subspecies, have conspicuous patches of pale setae on the ventro-lateral portions of T5 and T6. These same characters were also used to separate *X. v. texana* from *X. v. virginica* by both Ackerman (1916) and Hurd (1961), yet proved useful in this study only when combined with additional characters. The general occurrence of patches of pale setae on the last few segments of the metasoma of some *Xylocopa* suggests that setae color might be phenotypically plastic or have an adaptive explanation. Although poikilothermic, the *Xylocopa* exhibit some thermoregulatory ability, such as raising internal temperatures by thoracic flight muscle movement (Gerling, *et al.* 1989) or internal cooling by moving hemolymph from the thorax to the abdomen (Chappell 1982). Gerling, *et al.* (1989) hypothesized that variation in cooling rates among carpenter bee species was attributable to the degree of metasomal pubescence. Similarly, variation in the extent of black setae on the dorsum of the thorax of *X. virginica* might be linked to thermal regulation. Black bands of setae between the tegulae are also common among bumble bee species, and these are thought to aid in warming the thoracic flight muscles in low ambient temperatures (Williams 2007). The cause of setal color variation among *X. virginica* subspecies is unknown, but some geographic trends seem to

exist. Whether color variations are the fixed result of environmentally-driven local adaptation, the differential expression of phenotypic plasticity or the byproduct of evolutionary stochasticity among different lineages remains to be seen.

The distributions of each subspecies of *X. virginica* are not as clearly allopatric as claimed by Hurd (1961), and their morphologies are not as discrete. The subspecies *X. v. krombeini* is not morphologically diagnosable from the nominate subspecies *X. v. virginica* in either sex, nor is it geographically separated. However, the distinctiveness of *X. v. texana* and *X. v. virginica* suggests that the species has had a complicated evolutionary past. Prevailing hypotheses state that all *Xylocopa* in North America were pushed into Central and South America at the last glacial maximum around 12,000 years ago (Leys, *et al.* 2002). Perhaps at this point, the two lineages of *X. virginica* were isolated and began to diverge. A phylogeographic analysis of *X. virginica* subspecies should yield insights into this phenomenon.

An updated key to the subspecies of *X. v. virginica* is provided below, with each couplet starting with the most reliable character, followed by other, more variable ones. The variability observed in this study has been imbedded within this key so that practitioners might weigh multiple lines of morphological and geographic evidence before making a subspecies diagnosis for *X. virginica* specimens. Still, care should be taken, particularly when diagnosing worn specimens or those from intermediate zones as illustrated in Fig. VI.D.7. Not all specimens can be accurately assigned to subspecies.

F. A Revised Dichotomous Key to the Subspecies of *X. virginica*

1. Antennae with 11 flagellomeres; metasoma with seven visible terga, the last, T7, apically rounded and lacking a sting mechanism ..... MALES, 2

– Antennae with 10 flagellomeres; metasoma with six visible terga, the last, T6, pointed and concealing the sting mechanism .....FEMALES, 3

2 (1). Paraocular area entirely (Fig.VI.D.3.C) or partially (Fig.VI.D.3.B) black pigmented; dorsal apex of T4 often with an interrupted band of pale setae; metasoma often with greenish or bluish iridescence (Fig. VI.B.2.A) (typically west of about 95° longitude in parts of Texas, Oklahoma and Kansas) .....*texana* Cresson, 1872

– Paraocular area entirely without black pigmentation (Fig.VI.D.3.A); dorsal apex of T4 usually without an interrupted band of pale setae; metasoma usually matte black (Fig. VI.B.2.B) (typically east of about 95° longitude).....*virginica* (Linnaeus, 1771)

3 (2). Ratio of the height of the frontal carina to the distance from the carina to the antennal base greater than one (Fig. VI.B.2.G); length of tibial scale approximately equal to length, giving it a squarish appearance (Fig. VI.C.1.B); teeth of tibial scale approximately equal in length; metasoma often with greenish or bluish iridescence (Fig. VI.B.2.A) (typically west of about 95° longitude in parts of Texas, Oklahoma and Kansas).....*texana* Cresson, 1872

– Ratio of the height of the frontal carina to the distance from the carina to the antennal base less than one (Fig. VI.B.2.F); length of tibial scale approximately greater than to length, giving it an elongate appearance (Fig. VI.C.1.A); anterior tooth of tibial scale longer than posterior tooth; metasoma usually matte black (Fig. VI.B.2.B) (typically east of about 95° longitude) .....*virginica* (Linnaeus, 1771)

G. ACKNOWLEDGEMENTS

I wish to thank the following individuals for access to the specimens used in this work: Edward Riley (TAMU), James Wiley (FSCA), Jeffery Barnes (UAAM), Victoria Bayless and

Stephanie Gil (LSAM), Eugene Scarpulla for the large collection from Maryland, Jack Grubaugh, Xime Stewart, Barbara Lewis, Elizabeth Bergey and Karin Grimlund. I am also grateful to Jeffery Barnes for invaluable consultation during the preparation of this manuscript and to Clinton Trammel for photographs, collecting assistance and numerous insightful conversations on this subject. Collections were made under the following permits: Arkansas Natural Heritage Association (S-NHCC-12-013 and S-NHCC-13-010), Florida Fish and Wildlife Conservation Commission, Southwest Regional Office (SUO-49182), Louisiana Department of Wildlife and Fisheries (LNHP-13-048), permission from the Mississippi Department of Wildlife, Fisheries and Parks, Oklahoma Department of Wildlife (#5605) and Southwestern Florida Water Management District (#10-200). I am particularly grateful to Clinton Trammel for collecting assistance throughout the southeast and to Manny Lopez and Paul Elliot for assistance in finding specimens in Florida.

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Table VI.B.1. Morphological characters used to distinguish among *X. virginica* subspecies obtained from literature.

	X. v. virginica	*X. v. texana*	*X. v. krombeini*
Males			
Color of paraocular area integument	Pale yellow	Black	*
Pale pile present between antennae	No	Yes	*
Pale pile present on cheeks	No	Yes	*
Color of pile on dorsum of thorax	Yellow	Yellow mixed with black	Narrow ring of black
Color of metasomal integument	Black	Iridescent green or blue	Black
Pale pile on T4	No	Medially interrupted, subapical fringe	lateral patches (often)
Pale pile on T5	No	lateral patches	lateral patches
Pale pile on T6	No	lateral patches	lateral patches
Females			
Frontal carina size	Normal	Large	Normal
Color of pile on dorsum of thorax	Pure yellow	Black mixed with yellow	Pure yellow
Tibial scale shape	Elongated	As wide as long	*
Tibial scale teeth	Unequal	Even	*
Color of metasomal integument	Black	Iridescent green or blue	Black
Pale pile on T4	No	lateral patches (often)	lateral patches (often)
Pale pile on T5	No	lateral patches	lateral patches
Pale pile on T6	No	lateral patches	lateral patches

* Not explicitly described and assumed to follow *X. v. virginica*. T4, T5, T6 refer to metasomal terga; In carina size, "Large" indicates that the size of the carina is greater than the alveolocellar distance (Hurd, 1961) such that the median oculus appears sunken (Ackerman, 1916).

Table VI.C.1. Morphological characters, possible character states and coding used for analysis.

Group	Character	Possible States
Both Sexes		
	Metasoma- integument color	Black
		Iridescent
	Thorax- setae on dorsum	Pure yellow
		Mixed yellow and black
		Large black patch
	T4- lateral setae color	Black
		Few pale setae
		Large pale patch
	T4- apico-dorsal setae color	Black
		Pale
	T5- lateral setae color	Black
		Few pale setae
		Large pale patch
	T5- apico-dorsal setae color	Black
		Pale
	T6- lateral setae color	Black
		Few pale setae
		Large pale patch
Males		
	Clypeus- integument color	Pale
		Patterned pale and black
	Paraocular area- integument color	Pale
		Black
		Mixed pale and black
	Interanntenal setae	Black
		Mixed yellow and black
	Cheek setae	Pale
		Black
		Mixed yellow and black
Females		
	Frontal carina- height:breadth ratio	Numeric, continuous
	Tibial scale- shape	Elongate
		Square
	Tibial scale- comparative length of teeth	Posterior > anterior
		Equal

Table VI.D.1. Percent of individuals in each range exhibiting expected character states (in parentheses).

	Range of		
	X. v. virginica	*X. v. texana*	*X. v. krombeini*
Males			
Color of paraocular area	96 (pale)	88 (black)	100 (pale)
Pale pile present between antennae	59 (no)	80 (yes)	71 (no)
Pale pile present on cheeks	93 (no)	63 (yes)	100 (no)
Color of pile on dorsum of thorax	32 (yellow)	100 (some black)	100 (black ring)
Color of metasomal integument	93 (black)	60 (iridescent)	100 (black)
Pale pile on T4	74 (no)	37 (apical fringe)	50 (lateral patch)
Pale pile on T5	85 (no)	34 (lateral patch)	93 (lateral patch)
Pale pile on T6	93 (no)	25 (lateral patch)	93 (lateral patch)
Females			
Frontal carina size	99 (normal)	49 (large)	100 (normal)
Color of pile on dorsum of thorax	34 (yellow)	94 (some black)	21 (yellow)
Tibial scale shape	99 (elongate)	47 (square)	100 (elongate)
Tibial scale teeth	99 (unequal)	44 (equal)	100 (unequal)
Color of metasomal integument	96 (black)	40 (iridescent)	100 (black)
Pale pile on T4	84 (no)	29 (lateral patch)	50 (lateral patch)
Pale pile on T5	75 (no)	43 (lateral patch)	83 (lateral patch)
Pale pile on T6	98 (no)	14 (lateral patch)	12 (lateral patch)

Number of males in each range: 330, 65, 15 (9 excluded from overlapping ranges); number of females in each range: 348, 91, 24 (12 excluded from overlapping ranges).

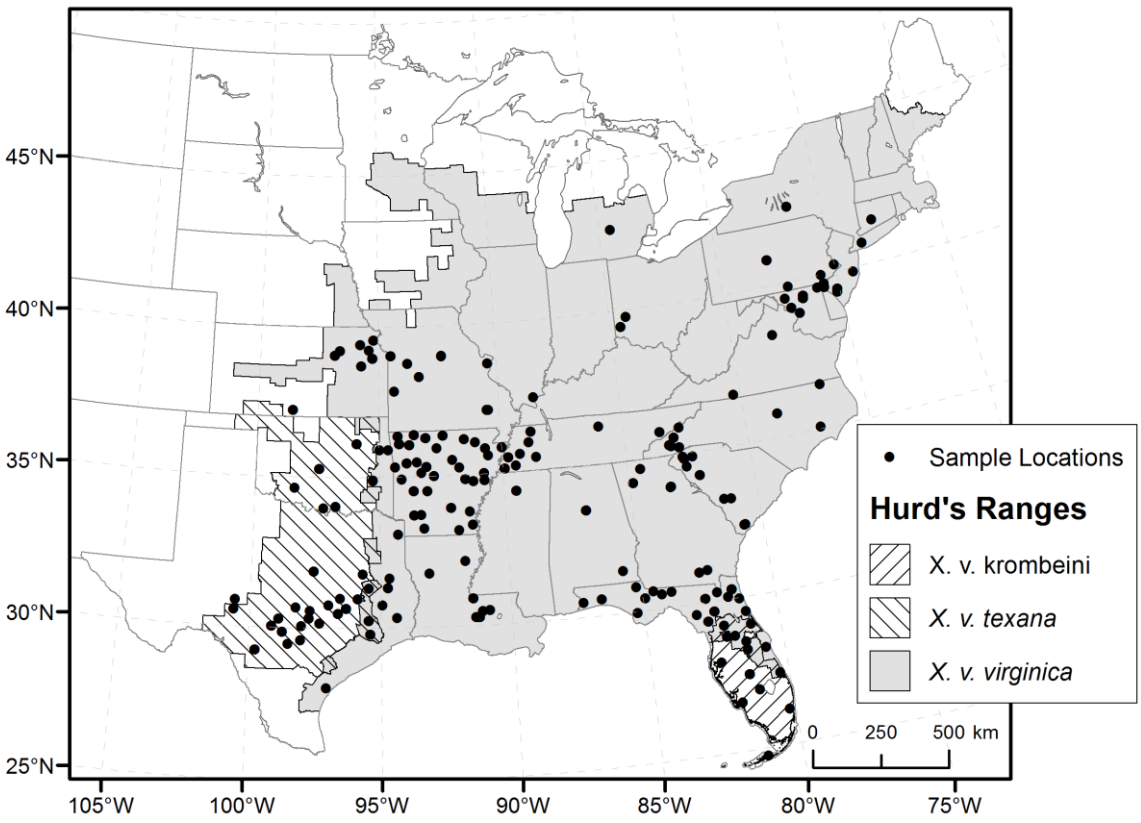

Figure VI.B.1. Map of the eastern United States showing the county-level historical distributions of *Xylocopa virginica* subspecies and sample locations for the specimens included in this study. Distributions were digitized from maps and data available in Hurd (1955) and Hurd and Moure (1963) using county-level locality data.

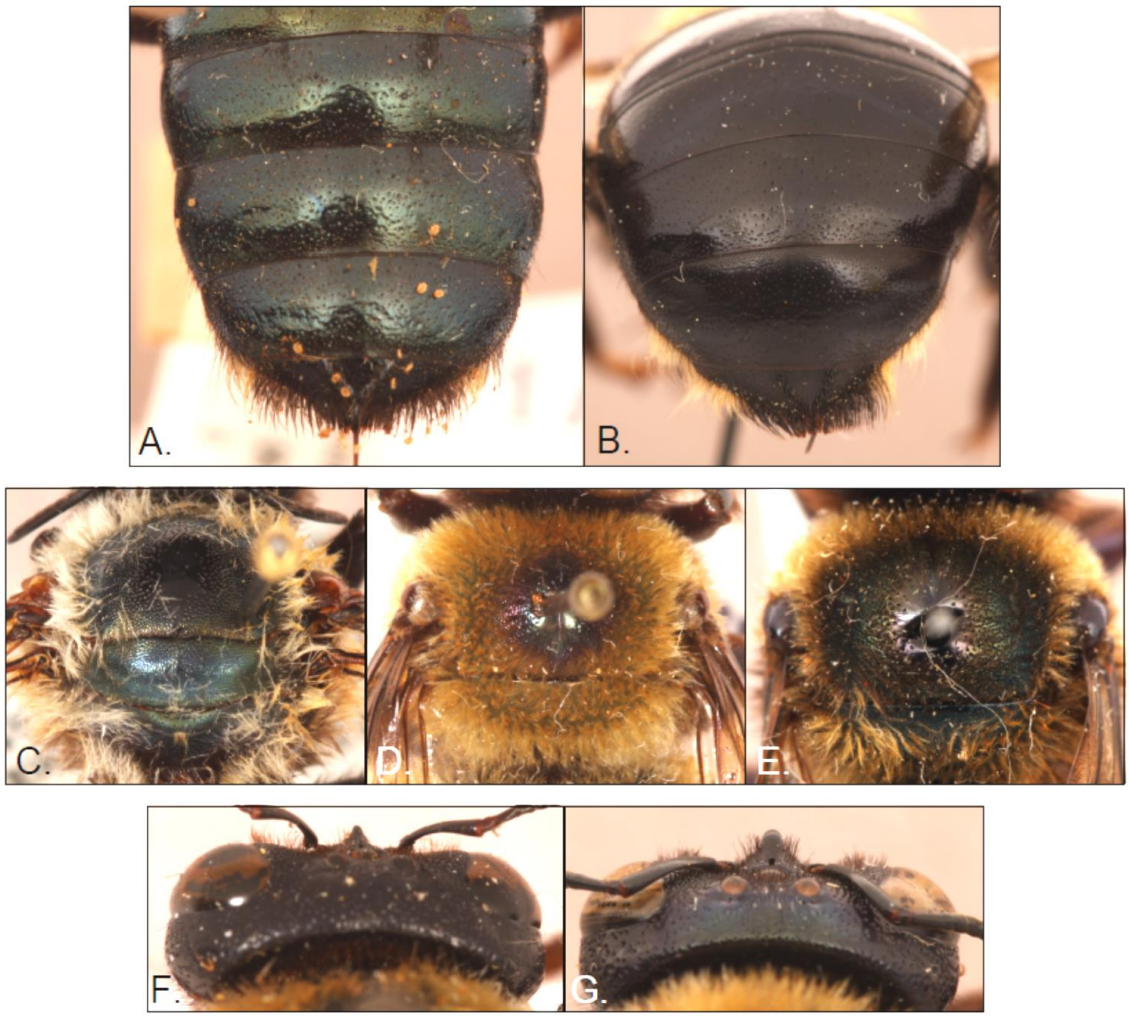

Figure VI.B.2.A–G. Photographs of *X. virginica* character states. A) Iridescent metasoma (*X. v. texana*); B) black metasoma (*X. v. virginica*); C) Dorsum of thorax worn; D) small black spot on dorsum of thorax; E) Large black spot on dorsum of thorax; F) small carina (*X. v. virginica*); G) large carina (*X. v. texana*). Photographs by Clinton Trammel, used with permission.

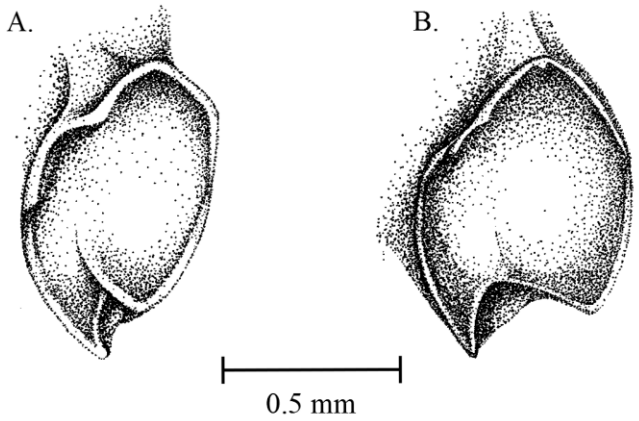

Figure VI.C.1.A–B. Tibial scale morphology of female *X. virginica.* A) elongate shape with the anterior tooth longer than the posterior tooth (*X. v. virginica*, 13-May-2010, St. Francis County, AR, UAAM) and B) square shape, with equally sized teeth (*X. v. texana*, 10-Oct-1975, Travis County, TX, TAMU). The scales of purported *X. v. krombeini* are identical to those of *X. v. virginica.*

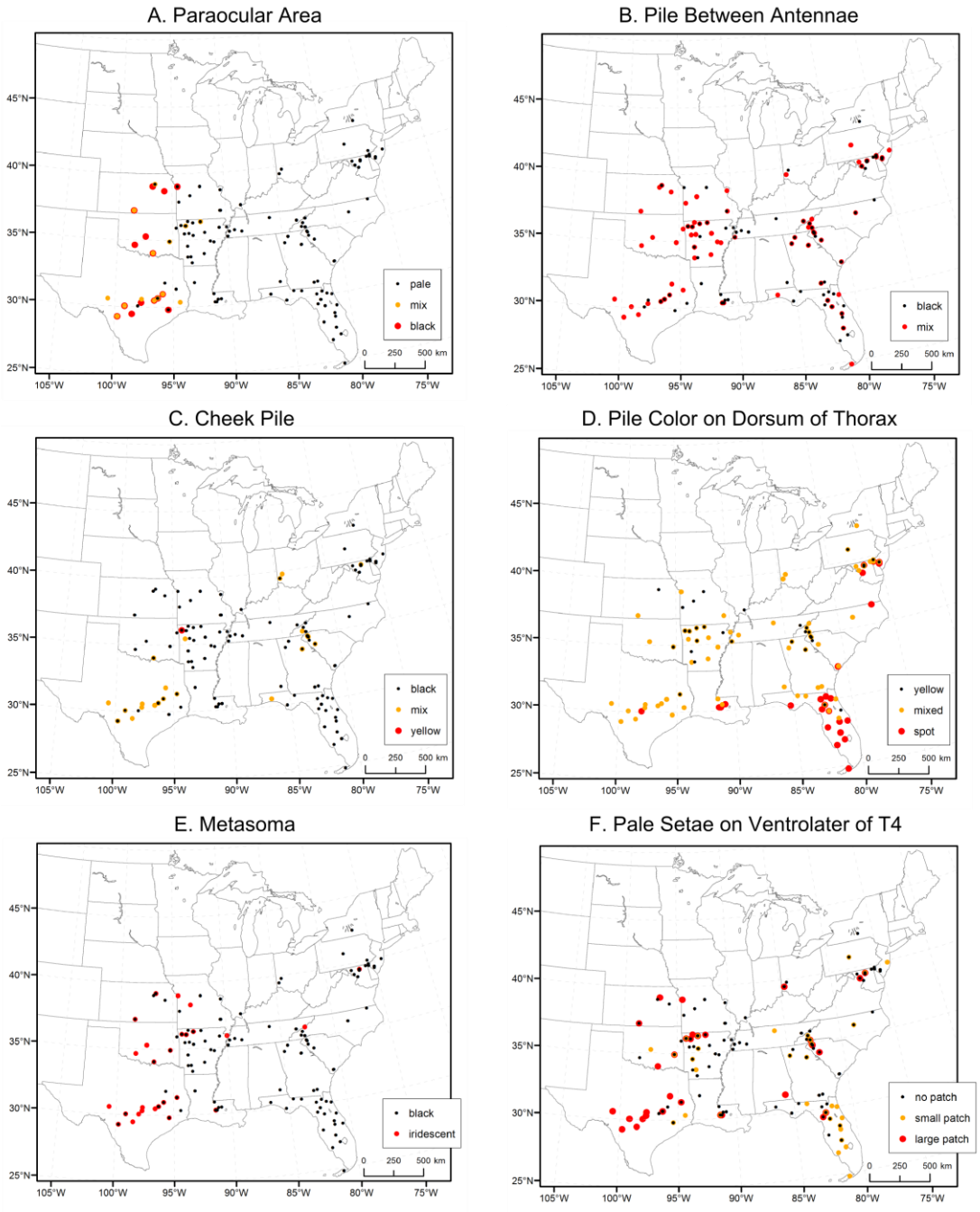

Figure VI.D.1 A–F. Distributions of character states scored for *X. virginica* males (n=422). In all cases, black dots represent states expected in the nominal subspecies, while gold and red dots indicate alternate states.

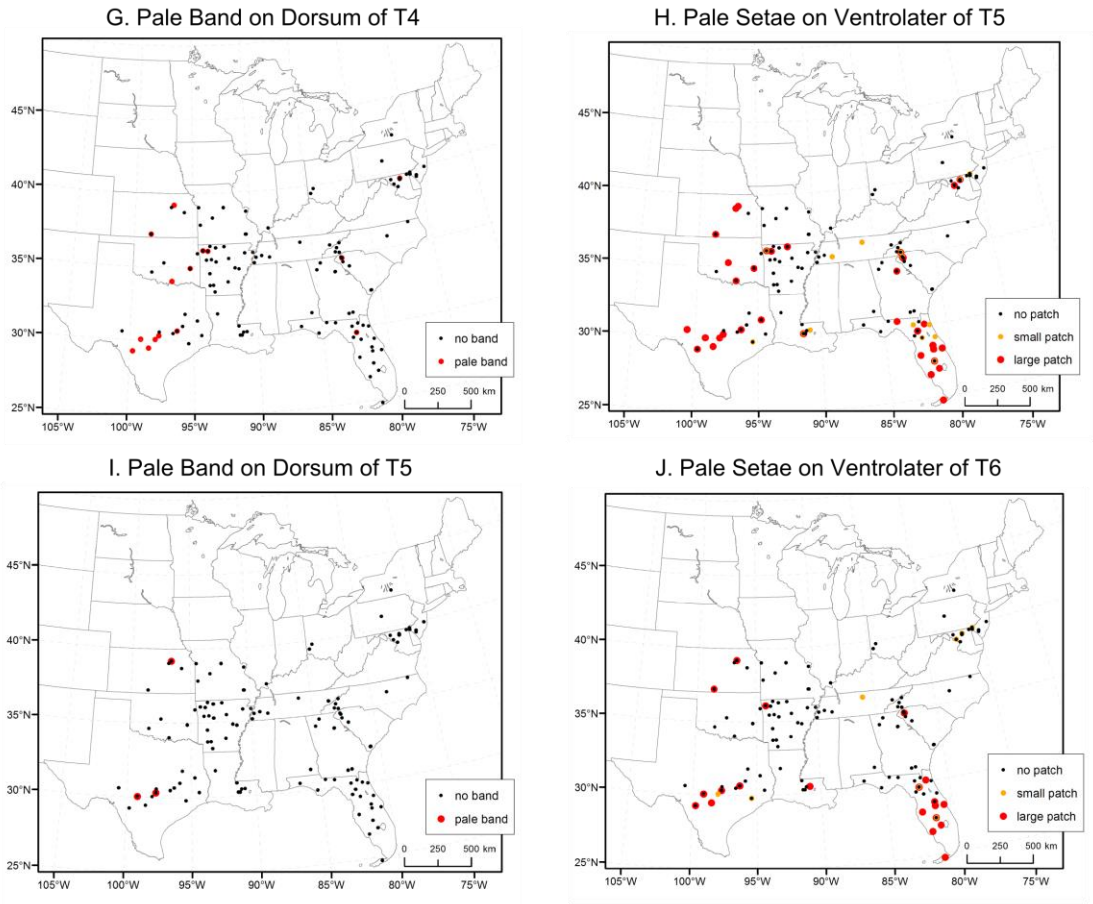

Figure VI.D.1 G–J. (Cont.)

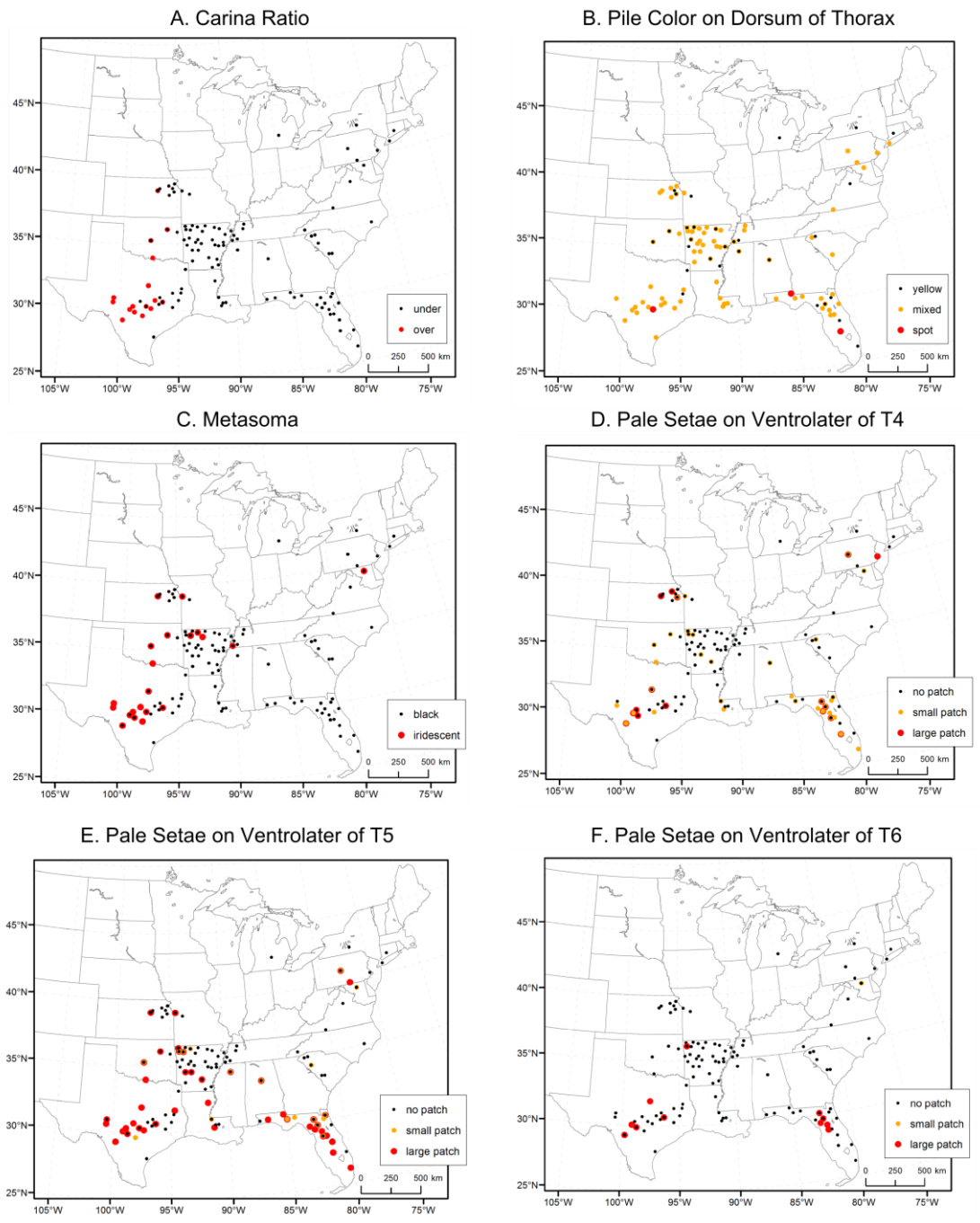

Figure VI.D.2 A–F. Distributions of character states scored for *X. virginica* females (n=477). In all cases, black dots represent states expected in the nominal subspecies, while gold and red dots indicate alternate states.

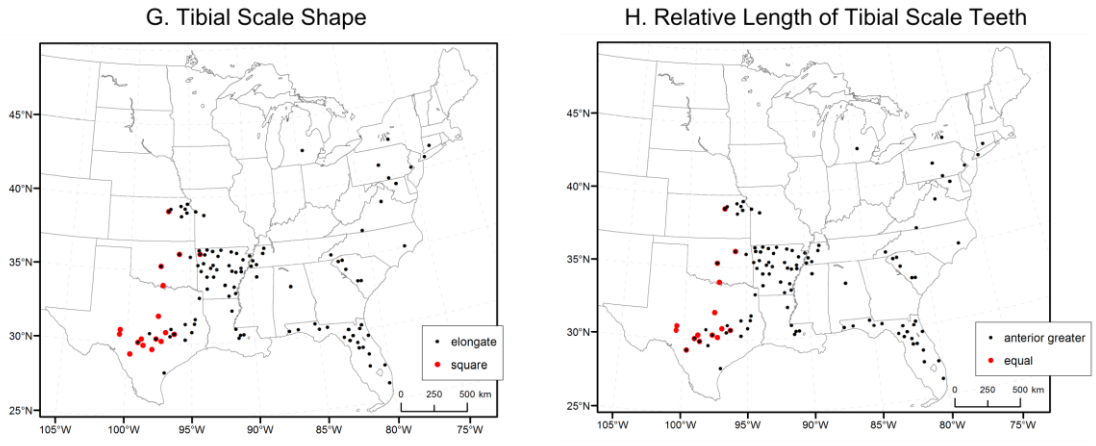

Figure VI.D.2 G–H. (Cont.)

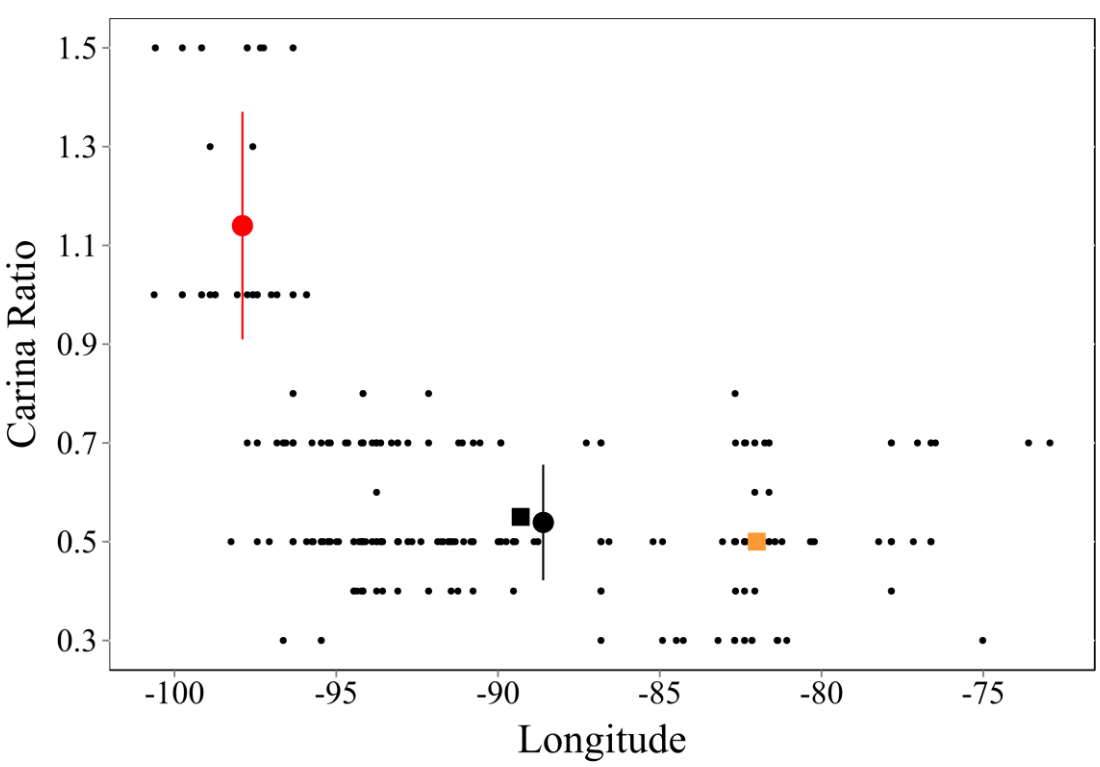

Figure VI.D.3. Relationship between the size of the carina (measured as the ratio between the height of the carina and the distance to the base of the antenna) and longitude. Larger dots indicate mean longitude and ratio for each subspecies (red=*X. v. texana* and black=*X. v. virginica*), and lines indicate standard deviations. Squares indicate means for the synonymized subspecies (gold=*X. v. krombeini* and black=*X. v. virginica*).

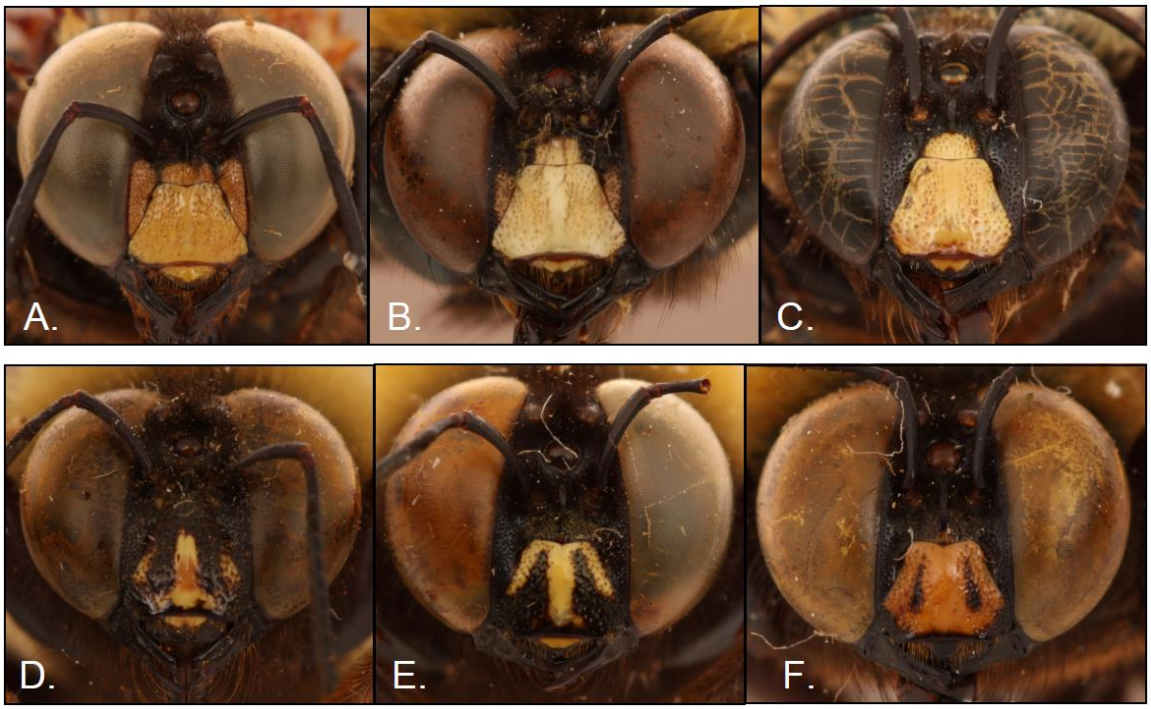

Figure VI.D.4.A–H. Photographs of paraocular area and clypeus coloration in *X. virginica* males. A) pale paraocular area (*X. v. virginica*); B) mixed paraocular area (*X. v. texana*); C) black paraocular area (*X. v. texana*); D–H) unusual clypeal color variation in Brazos County, Texas specimens (*X. v. texana*). Photographs by Clinton Trammel, used with permission.

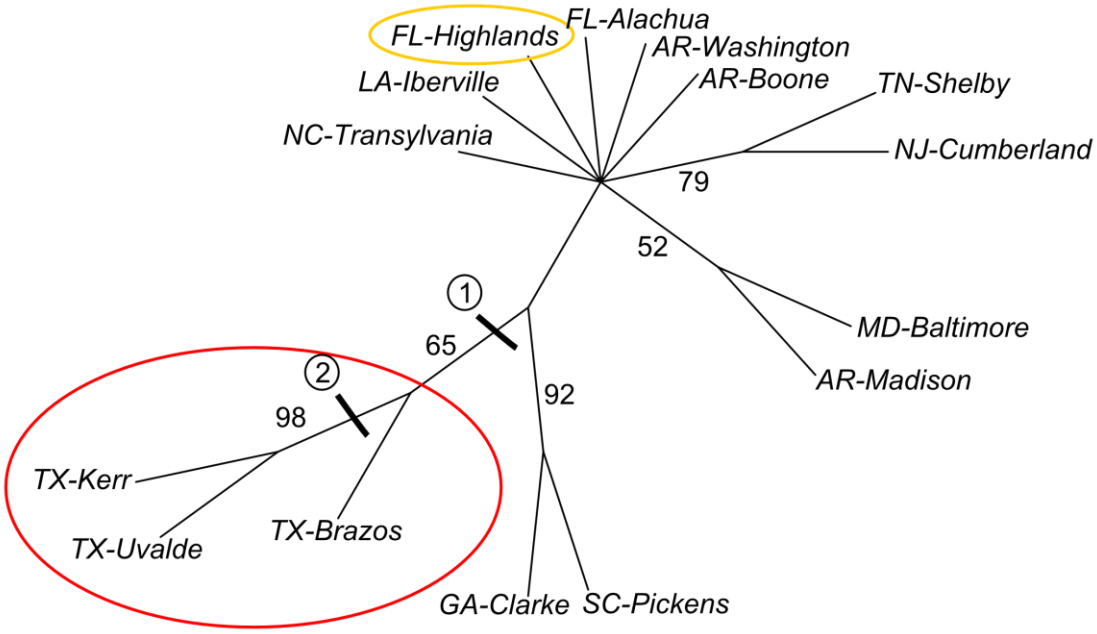

Figure VI.D.5. Unrooted Maximum Parsimony Majority Rule consensus cladogram of male morphology in 15 sampled populations. Consensus tree shows relationships recovered in 50% of the 26 trees with minimum length = 25 (CI=0.61; RI=0.63). Values at nodes are bootstrap estimates > 0.50 after 1,000 pseudoreplications. Numbered clades mark the following consistencies: 1) paraocular area black, 2) metasoma iridescent, T4 with dorso-apical fringe of pale setae, T4 and T5 with lateral, pale patches. Colored ellipses indicate Hurd's (1961) ranges of *X. v. krombeini* (gold) and *X. v. texana* (red). Tips without ellipses are in the range of the nominal subspecies.

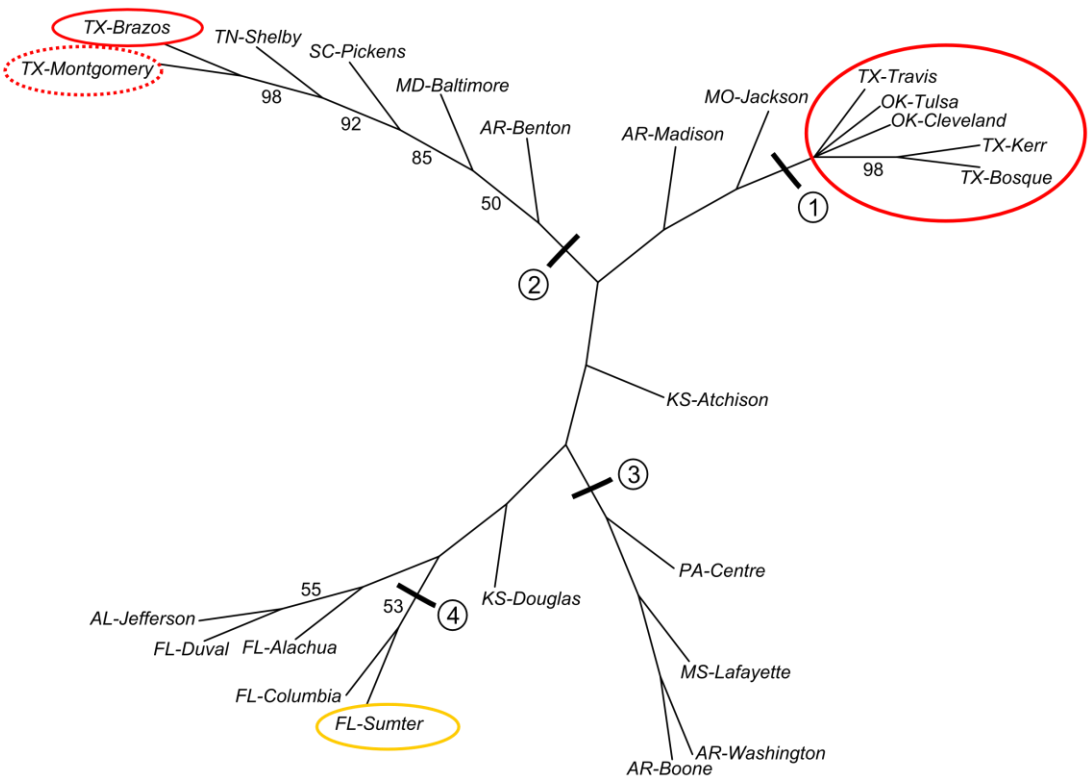

Figure VI.D.6. Unrooted Maximum Parsimony Majority Rule consensus cladogram of female morphology in 24 sampled populations. Consensus tree shows relationships recovered in 50% of the 2 trees with minimum length = 19 (CI=065; RI=0.80). Values at nodes are bootstrap estimates > 0.50 after 1,000 pseudoreplications. Numbers mark the following consistencies: 1) square tibial scale, 2) dorsum of the thorax: mixed with black, otherwise unambiguously matching *X. v. virginica* character states, 3) dorsum of the thorax: ambiguous, but otherwise unambiguously matching *X. v. virginica* character states and 4) dorsum of the thorax: mixed and T5 with pale patches. Colored ellipses indicate populations contained in the expected ranges of *X. v. krombeini* (gold) and *X. v. texana* (red); dotted lines indicate a range overlap between the indicated subspecies and *X. v. virginica*. Tips without ellipses are in the range of the nominal subspecies.

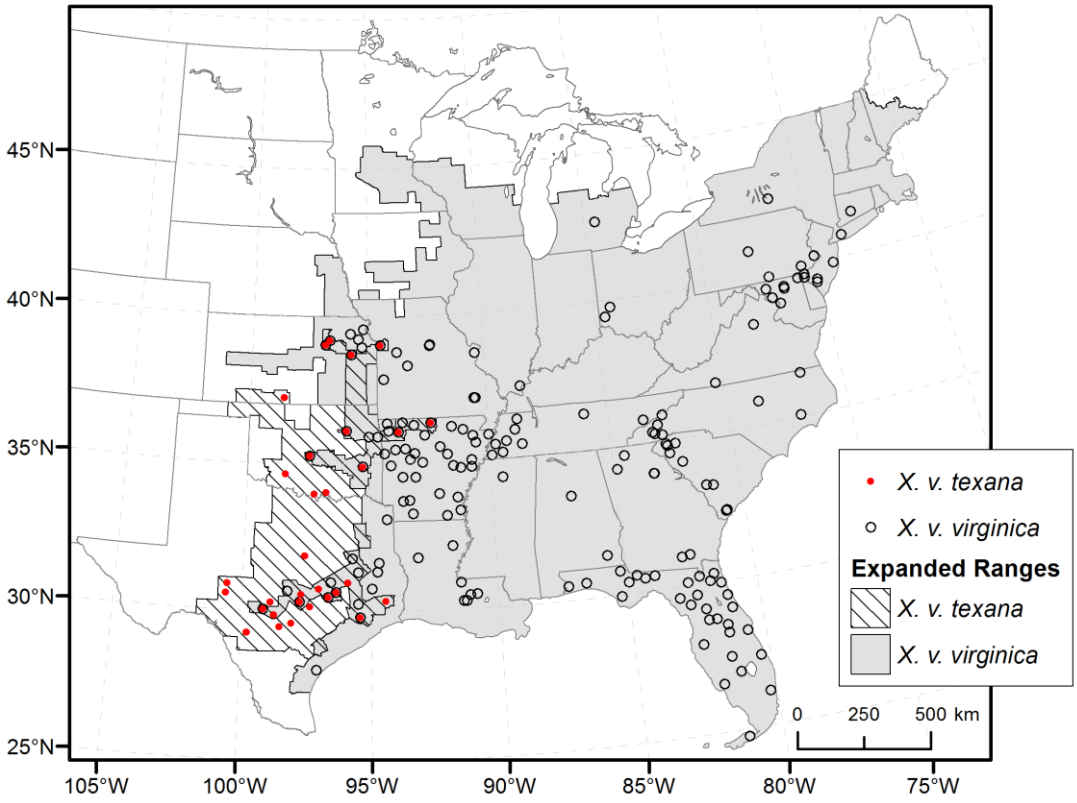

Figure VI.D.7. Map of the eastern United States showing subspecies diagnoses for the specimens included in this study: red dots=*X. v. texana,* open circles=*X. v. virginica* and expanded ranges for both (hatching=*X. v. texana,* grey=*X. v. virginica*) based on this work. Note that the subspecies *X. v. krombeini* was regarded as a junior synonym of *X. v. virginica.*

APPENDIX VI.I.1. Population profiles for males used in Maximum Parsimony analyses.

Population	Metasoma	Cheek	Paraocular	Interantennal	Thorax	T4-lateral	T4-dorsal	T5-lateral	T6-lateral
AR-Boone	±	black	pale	black	yellow	±	none	none	none
AR-Madison	±	black	pale	±	±	none	none	none	none
AR-Washington	black	black	pale	±	±	±	none	none	none
FL-Alachua	black	black	pale	±	±	±	none	none	±
FL-Highlands	black	black	pale	±	spot	±	none	patch	patch
GA-Clarke	black	±	pale	mixed	±	±	none	none	none
LA-Iberville	black	black	pale	black	spot	±	none	none	none
MD-Baltimore	black	black	pale	±	±	none	none	none	none
NC-Transylvania	black	black	pale	±	mixed	±	none	±	none
NJ-Cumberland	black	black	pale	black	mixed	none	none	none	none
SC-Pickens	black	±	pale	mixed	±	±	none	none	none
TN-Shelby	black	black	pale	black	mixed	none	none	none	none
TX-Brazos	±	±	black	mixed	mixed	±	none	none	none
TX-Kerr	iridescent	mixed	black	mixed	mixed	patch	pale	patch	patch
TX-Uvalde	iridescent	±	black	mixed	mixed	patch	pale	patch	±

Population sizes ranged from 5 to 82 (mean=18, SD=20). All characters were scored as binary states, with the exception of *Thorax*, which had three states, as described in the text and summarized in Table VI.C.1. The symbol ± indicates a character that was variable in the population with no single state present in > 25% of examined specimens.

APPENDIX VI.I.2. Population profiles for females used in Maximum Parsimony analyses.

Population	N	Metasoma	Carina	Thorax	T4-lateral	T5-lateral	T6-lateral	scale
AL-Jefferson	21	black	under	±	±	±	none	long
AR-Benton	6	black	under	mixed	none	none	none	long
AR-Boone	17	black	under	±	none	none	none	long
AR-Madison	11	±	under	mixed	none	none	none	long
AR-Washington	42	black	under	±	none	none	none	long
FL-Alachua	21	black	under	±	±	patch	none	long
FL-Columbia	9	black	under	mixed	±	patch	none	long
FL-Duval	18	black	under	±	±	±	none	long
FL-Sumter	21	black	under	mixed	±	patch	none	long
KS-Atchison	5	black	under	yellow	none	none	none	long
KS-Douglas	7	black	under	±	±	none	none	long
MD-Baltimore	27	black	under	mixed	none	none	none	long
MO-Jackson	6	±	under	mixed	none	±	none	long
MS-Lafayette	10	black	under	±	none	none	none	long
OK-Cleveland	8	±	±	mixed	±	±	none	square
OK-Tulsa	5	black	over	±	none	±	none	square
PA-Centre	17	black	under	±	none	none	none	long
SC-Pickens	9	black	under	mixed	none	none	none	long
TN-Shelby	13	black	under	mixed	none	none	none	long
TX-Bosque	5	iridescent	over	mixed	patch	patch	patch	square
TX-Brazos	42	black	under	mixed	none	none	none	long
TX-Kerr	5	iridescent	over	mixed	patch	patch	±	square
TX-Montgomery	7	black	under	mixed	none	none	none	long
TX-Travis	5	iridescent	over	mixed	none	±	none	square

Populations are noted with a two letter abbreviation for the state, followed by the county name. N=number of specimens. Characters are scored as described in the text and summarized in Table VI.C.1. The symbol ± indicates a character that was variable in the population with no single state present in >25% of examined specimens.

VII. PHYLOGEOGRAPHY OF *XYLOCOPA* LATREILLE (HYMENOPTERA: APIDAE) IN EASTERN NORTH AMERICA: POST-GLACIAL PATTERNS OF COLONIZATION

A. ABSTRACT

Although they have similar ecological niches, the two species of large carpenter bees (Hymenoptera: Apidae: *Xylocopa*) in eastern North America have very different distributions; *Xylocopa virginica* (Linnaeus, 1771) is widespread, yet *X. micans* Lepeletier, 1841 has a limited distribution. Contrary to leading hypotheses, phylogeographic analyses with a mitochondrial marker suggest that their post-glacial colonization patterns differ. Both species showed signals of recent demographic expansion, but *X. virginica* additionally showed isolation by distance and population structuring in the eastern portion of its range. The distribution of *X. virginica* haplotypes suggests that this species expanded from multiple refugia, including at least one west and one east of the Mississippi River, and likely more than one in the east as evidenced by greater diversity (H_d = West: 0.59±0.05; East: 0.81±0.01) and higher pairwise fixation indices (average Φ_{ST} = West: 0.07±0.12; East: 0.32±0.30) in this region. On the other hand, *X. micans* haplotype distributions are consistent with a single origin, likely west of the Mississippi River. In spite of its interpopulation homogeneity, *X. micans* is genetically quite diverse compared to *X. virginica* (H_d = 0.91±0.03 and 0.78±0.02, respectively). This is consistent with Hewitt's leading-edge hypothesis for range disparity, which posits that species in more northerly refugia have an ecological advantage during initial recolonization, and block the advance of species with similar requirements residing farther south.

B. INTRODUCTION

The large carpenter bees (*Xylocopa* Latreille, 1802) (Hymenoptera: Apidae) are generalist pollinators whose worldwide distribution is thought to be governed by historical

biogeography (Leys, *et al.* 2002) and climatic factors such as temperature and precipitation (Porter 1981; Watmough 1983). Including *Lestis* Lepeletier and Serville, 1828 and *Proxylocopa* Hedicke, 1938 as subgenera (Minckley 1998), there are 470 *Xylocopa* species subdivided into 51 subgenera worldwide (Hurd and Moure 1963). Most *Xylocopa* are distributed throughout the tropics and subtropics (Leys and Hogendoorn 2008), but two species occur in eastern North America: *Xylocopa* (*Schonnherria*) *micans* Lepeletier, 1841 and *X.* (*Xylocopoides*) *virginica* (Linaeus, 1771).These species co-occur in the southern part of North America, but they exhibit widely different range sizes (*X. micans:* Fig. VII.B.1; *X. virginica:* Fig. VI.D.7). *Xylocopa virginica* has a wide distribution east of the 100th meridian, with a range that extends northward from the Gulf of Mexico into Ontario, Canada (Hurd 1955; Skandalis, *et al.* 2011). On the other hand, the distribution of *Xylocopa micans* is much smaller and limited to the southeastern United States along the Atlantic coast (Hurd 1955). Recent records also indicate that *X. micans* might be expanding its range northward (Warriner 2010; Tripodi and Szalanski 2011).

The subgenera *Xylocopoides* and *Schonnherria* apparently diverged and independently colonized North America after crossing Beringia over 34 million years ago (Leys, *et al.* 2002). By examining their morphology and preferred nesting sites, Hurd (1956) proposed that the *Schonnherria* were present in the New World prior to the arrival of *Xylocopoides*. After being pushed south at the last glacial maximum (LGM), they might have independently recolonized North America from Central America as the glaciers receded and temperatures rose about 12,000 years ago (Leys, *et al.* 2002). However, recent evidence suggests that although temperatures were much lower, multiple refugia might have been present in unglaciated southeastern North America (reviewed in Soltis, *et al.* 2006) that could have provided suitable habitat for carpenter bees. The Wisconsin glaciation covered northern North America in permafrost and ice south to

about 39° latitude at the LGM approximately 18,000 to 21,500 years ago (Soltis, *et al.* 2006). Although the eastern portion of North America remained largely unglaciated during the LGM, temperatures were an estimated 10–20°C colder than modern day regional temperatures (Jackson, *et al.* 2000), and many taxa either moved southward or were extirpated. The exact locations of the southerly refugia are largely unknown for many taxa, however. In a synthesis of Nearctic insect distribution and phylogenetic patterns, Ross (1953) hypothesized that insects in eastern North America would not have moved farther southwest than the Ozark Plateau (Missouri and Arkansas) or the Texas Cross Timbers (Central Plains of Oklahoma and Texas). The Florida peninsula was a refuge for some insect taxa, although summer temperatures there were equivalent to those in Michigan today, with an estimated mean July temperature of about 18.5°C and mean January temperature of about 1.7°C (Howden 1969). Additional refugia have been proposed for other taxa in the Lower Mississippi Valley, the Ozarks and the southern Appalachian mountains (Soltis, *et al.* 2006).

As the glaciers receded and climatic conditions became more suitable, initial colonists would have dispersed quickly from more northerly refugia. Populations of initial colonists in the large, previously glaciated areas of the north would expand quickly into suitable ecological niches and occupy large geographic ranges. In contrast, secondary colonists with similar resource needs that survived the glaciations much further south would find it difficult to occupy these already inhabited areas. These taxa should exhibit smaller geographic ranges but have higher genetic diversity and potentially greater genetic structure among populations (Hewitt 2000; Douglas, *et al.* 2009). The contemporary distributions of *Xylocopa* in the eastern United States show a pattern that suggests that *X. virginica* could have been an initial colonizer such as this, followed secondarily by *X. micans*.

On the other hand, *X. virginica* is a polytypic species, with two accepted subspecies: *X. v. texana* Cresson, 1872 and *X. v. virginica* (Linnaeus, 1771) (Hurd 1961). A third, *X. v. krombeini* Hurd, 1961 was also described but morphological analyses suggest that it is unlikely to be a valid subspecies (see Chapter VI). The morphological diversity and geographic distributions of the subspecies of *X. virginica* suggests that the species might have greater genetic diversity and population structure than the monomorphic *X. micans*. The diversity in *X. virginica* subspecies could be attributable to the persistence of disparate populations isolated in separate refugia and experiencing different evolutionary trajectories. This has been seen in other insect taxa (*e.g. Magicicada,* Davis, 1925 (Hemiptera: Cicadidae); *Pselaphinae,* Latreille, 1802 (Coleoptera: Staphylinidae); *Allocapnia,* Claassen, 1928 (Plecoptera: Capniidae); and *Osmoderma,* LePeletier and Serville, 1828(Coleoptera: Scarabaeidae)) derived from ancestors that survived just south of the glacier's extent in separate refugia (reviewed in Howden 1969). The polymorphism of *X. virginica* in the eastern United States suggests the presence of independent lineages of this species, perhaps as a result of isolation during the LGM. If these subspecies are independently derived, the histories of their lineages should exhibit a recognizable phylogeographic signal. Phylogeographic methods allow genetic histories to be linked to geographic distributions (Avise 2009). Phylogenetic and demographic analysis of species throughout their ranges can address colonization hypotheses, and comparative analysis of similar, related taxa might offer insight to the factors that govern past, current and future distributions (Parmesan, *et al.* 2005).

Objectives

There are three generalized hypotheses proposed to describe the post-LGM recolonization of eastern North America by *Xylocopa.* The first is that the ancestors of both groups recolonized North America from Central America after the ice receded (Leys, *et al.*

2002). This would yield a genetic signal of a single source in each species as they expanded northeastward from current day southern Texas to fill the remainder of their ranges. An alternative hypothesis is that some parts of North America would have remained suitable during the LGM, and populations could have expanded from multiple refugia. This would yield a phylogeographic signal of genetic differentiation among populations of each species, indicative of multiple source refugia. An additional hypothesis, which could accompany either of the former hypotheses, follows from Hewitt's (2000) initial-colonizers scenario. This final hypothesis proposes that the range disparity between *X. virginica* and *X. micans* can be explained by *X. virginica* persisting at more northerly refugia than *X. micans,* giving *X. virginica* the ecological advantage during initial recolonization of eastern North America. This hypothesis would be supported by a lower genetic diversity within *X. virginica* and greater signals of population expansion than exhibited by *X. micans.*

C. MATERIALS AND METHODS

Specimen Acquisition and Identification

Most specimens for genetic analysis were collected between 2010 and 2013 via aerial net and stored in 95% ethanol, but four pinned specimens dated 2006–2009 from the University of Arkansas Arthropod Museum (UAAM) were also included as were 26 pinned specimens from Maryland (2011) and nine from Tennessee (2010-2012). During sampling, latitude-longitude coordinates were determined using the built-in Compass application on an iPhone 3GS (Apple, Cupertino, CA). These were subsequently verified by viewing coordinates on Google Maps (maps.google.com, Google, Mountain View, CA) and manually adjusting them according to stable landmarks (*e.g.* roads) witnessed at the site (*X. micans:* Fig.VII.C.1, *X. virginica:* Fig.VII.C.2). Each specimen was identified to species using the morphological key of Hurd

(1955). Voucher specimens have been deposited in UAAM. Distribution data for *X. micans* were also obtained from specimens in the Florida State Collection of Arthropods (FSCA) and Texas A&M University Insect Collection (TAMU) and collection data available on the Global Biodiversity Information Facility (GBIF, http://data.gbif.org, accessed 12-Dec-2013). Observational data were culled, and only records of specimens deposited within museums were retained (Fig. VII.B.1).

Genetic Methods

Total genomic DNA was extracted from either a single mid-leg or one-half of a thorax from individual specimens using the salting-out procedure (Sambrook and Russell 2001) described in Chapter III. Genetic vouchers are housed at the Insect Genetics Laboratory at the University of Arkansas. PCR was conducted using the barcoding primers LCO-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer, *et al.* 1994), which amplify a 709 bp portion of the cytochrome-oxidase subunit I (*COI*) region of the mitochondrial genome. PCR reactions consisted of 2 µl DNA extraction, 5 µl 10x reaction buffer (Thermopol, New England BioLabs, Ipswich, MA), 4 µl nucleotides (10 mM, dATP, dTTP, dCTP and dGTP), 1 µl each primer (20 µM), 2 units *Taq* polymerase (New England BioLabs, Ipswich, MA) and ultrapure water for a total 50 µl volume. Both a positive control (2 µl DNA extraction from a sample that had been successfully amplified and sequenced in the past) and a negative control (2 µl ultrapure water in place of DNA extraction) were included in each PCR batch. Reaction conditions were 94ºC for 2 min, followed by 40 cycles of 94ºC for 45 s, 53ºC for 1 min, and 72ºC for 1 min, with a final 5 min extension step at 72ºC. Amplified DNA was subjected to electrophoresis in a 2% agarose gel along with a 100 bp reference ladder (New England BioLabs, Ipswich, MA), and

stained with ethidium bromide. Amplicons were then visualized under ultra-violet light (BioDoc-it, UVP, CA) to assess PCR success. Amplicons which produced bands at around 700 bp were deemed successful and prepared for sequencing. Amplified DNA was purified and concentrated with PES 30k centrifugal filter devices (VWR, Radnor, PA), then sent for direct sequencing in both directions (University of Arkansas Medical School, Little Rock, AR or Eurofins MWG Operon, Huntsville, AL).

Sequence Alignments and Haplotype Designations

The forward and reverse sequences obtained for each sample were aligned to one another using the alignment tool within GENEIOUS v6.1.6 (Kearse, *et al.* 2012) with a 65% similarity cost matrix, a gap open penalty of 12, a gap extension penalty of three and four refinement iterations. For each sample, a consensus sequence was determined and the primer regions were trimmed, leaving a 658 bp sequence. Each consensus sequence was compared to a running list of haplotypes recovered from *Xylocopa* samples in this study. A sequence exhibiting one or more nucleotide differences not previously observed was deemed a new haplotype and added to the running list of haplotypes for each species. Because *COI* is a protein-coding region, the translation tool within GENEIOUS (frame 2, invertebrate mitochondrial code) was employed to determine non-synonymous nucleotide substitutions resulting in protein changes in new haplotypes and ensure that such changes did not result in the introduction of stop codons.

Analysis of Genetic Relationships

Characteristics of the genetic data were estimated with ARLEQUIN v.3.5.1.2 (Excoffier and Lischer 2010). Simple linear regression was used to determine the effect of sample size on haplotypic diversity (H_d) and the number of haplotypes (Schneider, *et al.* 2010) using all

populations with a sample size greater than five. The MR. BAYES plug-in v. 3.2.1 (Huelsenbeck and Ronquist 2001) within GENEIOUS (Kearse, *et al.* 2012) was used to create a Bayesian inference of relationships using appropriate models of substitution as determined with corrected Akaike information criterion in JMODELTEST v.2.1.4 (Darriba, *et al.* 2012). All unique haplotypes of *X. micans* and *X. virginica* were used as ingroup taxa, with four close relatives as outgroup taxa (Apidae: *Bombus auricomus, B. bimaculatus* and *B. fraternus;* Megachilidae: *Megachile sculpturalis*). All unique *COI* sequences generated for this study were deposited in Genbank (Accession numbers KM585611–KM585687). Alignments between all sequences were unambiguous and no indels or stop codons were observed. Bayesian analysis was performed with a chain length of 1,100,00, four heated chains, a 100,000 burn-in length and trees were subsampled every 200 trees.

Because intraspecific phylogenies rarely result in cleanly bifurcated trees (Posada and Crandall 2001), network approaches were also undertaken to show relationships among haplotypes. Networks are useful for illustrating uncertainty in relationships among haplotypes when the data do not bifurcate in a tree-like manner. Network models account for uncertainty by allowing mutifurcations, (*e.g*. minimum spanning trees) and illustrating conflicting signals among potential haplotype relationships (*e.g.* neighbor net diagrams) (Bryant and Moulton 2004; Huson and Bryant 2006). Minimum spanning trees for each species were constructed using statistical parsimony with 95% confidence using the *pegas* package v.0.5.1 (Paradis 2010) within R v.3.1.0 (R Core Team 2014). Relative sample sizes of each haplotype were illustrated by adjusting the size of the circle representing each haplotype by the square root of the number of individuals with that haplotype. Under coalescent theory, the most frequently encountered haplotype is likely the oldest (Donnelly and Tavaré, 1986), and ancestral haplotypes are more

likely to be geographically widespread and exhibit multifurcations than more recently derived haplotypes (Posada and Crandall 2001). Ancestral haplotypes were surmised using these criteria. Because minimum spanning trees only represent a single tree out of many possibilities, they are a simplification of the underlying complexity in non-tree like networks. Therefore, neighbor net splits diagrams were used as an additional technique to explore uncertainty in these data using uncorrected p-distances in SPLITSTREE v.4.13.1 (Huson and Bryant 2006). If the reader is unfamiliar with these diagrams, Appendix VII.I.1 is provided to aid in their interpretation.

Analysis of Population Patterns

Analyses at the population level were conducted by grouping all collections within a single county or province as a single population, resulting in 55 populations of *X. virginica* and 14 populations of *X. micans*. Examination of *X. virginica* sample locations revealed that a single region had been vastly oversampled relative to the rest of the species' range (Fig. VII.C.2.A–B). To account for this in population-level spatial analyses, the samples in the 16 sites in the oversampled region were randomly resampled without replacement to reduce the dataset in this area to one-half of its total. This reduced the dataset to 52 populations and 304 individuals (Fig. VII.C.2.C–D). Populations were grouped in some analyses by *a priori* hypotheses: 1) an east-west division marked by the Mississippi River for both species and 2) typical range versus expanded range for *X. micans.* Isolation by distance was analyzed for all populations with n>1, resulting in 39 populations of *X. virginica* (reduced set) and eight populations of *X. micans.* Pairwise geographic distances were calculated using the Geographic Distance Matrix Generator v.1.2.3 (Ersts 2014), and coupled with pairwise genetic distances (estimated with Φ_{ST} values) to examine isolation by distance using the program IBDWS v.3.23 (Jensen, *et al.* 2005). A Mantel test was performed with 10,000 randomizations to determine significance. Isolation by distance

analysis was performed in three batches for each species: one for the entire dataset, one for the populations east of the Mississippi River (*X. virginica*=19 populations, *X. micans*=four populations) and one for populations west of the Mississippi River (*X. virginica*=20 populations, *X. micans*=four populations). Population structuring was assessed in both species using Analysis of MOlecular VAriance (AMOVA) using fixation indices ($\Phi_{ST,}$ $\Phi_{SC,}$ Φ_{CT}) estimated from pairwise differences as implemented in ARLEQUIN v.3.5.1.2 (Excoffier and Lischer 2010). Covariance components and fixation indices were tested using 16,000 permutations to assess significance. As with the isolation by distance tests, each species was analyzed as a whole, then separately comparing populations east and west of the Mississippi River. An additional AMOVA test was conducted comparing the populations within the known distribution of *X. micans* to those that have been newly discovered north of the known range.

D. RESULTS

Xylocopa micans Distribution

A total of 248 *X. micans* specimens were morphologically examined and georeferenced, including 116 from the TAMU collection (1917–1996), 48 from the FSCA collection (1939–2008), 3 from the UAAM collection (2006–2007) and 81 collected for this project (2010–2013) (Fig. VII.B.1). An additional 79 *X. micans* specimen records were available from the collections databased in GBIF (1888–1996). Five GBIF records were located outside of the range of *X. micans* as indicated by Hurd (1955): one from 1888 in Carlinville, Illinois (Macoupin County, 655 km from range edge), one in Benoit, Mississippi (Bolivar County, 71 km from range edge) from 1955 and three near Brady, Texas (McCulloch County, 86 km from range edge) with no associated year. Three specimens from the UAAM collection were collected in Arkansas (Clark, Hempstead and Little River Counties), which were 163, 138 and 100 km outside of the known

range of *X. micans.* Twenty specimens collected for this work were also located outside of the known distribution of *X. micans* between 2010 and 2013, including five from Hazen, Arkansas (Prairie County, 208 km from range edge), 14 from Oxford, Mississippi (Lafayette County, 136 km from range edge) and one collected near Brownsville, Tennessee (Haywood County, 242 km from range edge).

Genetic Diversity of *Xylocopa virginica*

A total of 382 *X. virginica* specimens from 79 sites located in 14 US states (n counties=54) and one Canadian province were characterized for a 658 bp portion of *COI* (Fig. VII.C.2.A). Alignments among sequences were unambiguous and no gaps or frame shifts in the protein translation were observed. Nucleotide composition was T-A biased, with 11% C, 44% T, 34% A and 11% G. Thirty-five polymorphic nucleotide positions were observed (*S*=35), resulting in 38 haplotypes (*H*=38), of which 26 were singletons. No single nucleotide position exhibited more than two nucleotides (η=*S*). There were four non-synonymous mutations (in haplotypes XV33, XV35, XV36 and XV38), and the transition-to-transversion ratio was 34:1. Percent divergence ranged from 0.1 to 0.9%. The mean number of pairwise differences (π) was 1.36±0.84, and the average nucleotide diversity (π_n) was 0.0021±0.001 (Table VII.D.1). The haplotypic diversity (H_d) of the entire sample was 0.78±0.02. Tajima's *D* statistic was negative and significant (*D*=-2.04, *p*=0.001) as was Fu's *Fs* statistic (*Fs*=-28.11, *p*<0.0001). Regression analysis indicated that sample size did not affect the estimate of haplotypic diversity (*F*=0.22, *df*=1,25, *p*=0.64), but that it did affect the number of haplotypes found (*F*=23.5, *df*=1,25, *p*<0.0001).

The region incorporating Northern Arkansas and Western Tennessee was disproportionately sampled, relative to the remaining sample regions (n=158, 41% of total samples; Fig. VII.C.2.B), and was subjected to random subsampling to halve the data from this region (Fig. VII.C.2.C– D). Three haplotypes were lost in the randomized subsampling procedure (XV15 (n=1), XV21 (n=3) and XV43 (n=1)). In the process, the maximum sample size from any one site was reduced from n=43 (Washington Co., Arkansas) to n=26 (Baltimore Co., Maryland), and the average sample size dropped from 7 to 6 per site. The reduced dataset included 24 singleton haplotypes. Estimates of diversity and expansion remained relatively unchanged in the reduced dataset (Table VII.D.1). Regression analysis on the reduced dataset also suggested that sample size did not affect haplotypic diversity (F=0.002, df=1,20, p=0.97), but that it did affect the number of haplotypes found (=4.8, df =1,20, p<0.04). Six haplotypes were common (XV1: n=49, XV2: n=13, XV6: n=125, XV7: n=13, XV17: n=35, XV18: n=22) and accounted for 80% of the data (Fig. VII.D.1).

Genetic Diversity of *Xylocopa micans*

A total of 73 *X. micans* specimens from 16 sites in seven US states (n counties=14) were characterized with the same *COI* region. Alignments were unambiguous, with no gaps or frame shifts observed. Nucleotide composition was 11% C, 45% T, 29% A and 15% G, and the transition-to-transversion ratio was 13:2. Thirty five haplotypes (H=35) were observed, 24 of which were singletons. One site, position 34, exhibited three nucleotides, resulting in η=30 and S=29 for the set. One mutation was non-synonymous, in haplotype XM14. Percent divergence ranged from 0.2–0.9%. The mean number of pairwise differences (π) was 2.11±1.19, and the average nucleotide diversity (π_n) was 0.0032±0.002 (Table VI.D.1). The haplotypic diversity (H_d) of the entire sample was 0.91±0.03. Both Tajima's D and Fu's Fs statistics were negative

and significant (D=-2.04, p=0.004, Fs=-27.22, p<0.0001). Regression analysis indicated that sample size did not affect the estimate of haplotypic diversity (F=0.40, df =4, p=0.49), but that it did affect the number of haplotypes found (F =43.3, df =4, p=0.003). Five haplotypes were common (XM2: n=3, XM4: n=21, XM6: n= 4, XM8: n=3, XV13: n=6) and accounted for 51% of the data (Fig. VII.D.2).

Estimates were also made for the subset of *X. micans* specimens collected outside of its known range (n=14, five populations from Arkansas, Tennessee and northern Mississippi; grey dots, Fig. VII.B.1). Ten haplotypes (H=10) were observed in this group, three of which were singletons (XM7, XM30 and XM31), and two of which were only observed in this subsample (XM2: Prairie County, Arkansas and XM7: Little River County, Arkansas). Haplotypic diversity (H_d) of this group was 0.95±0.05, with π=1.18±1.11 (Table VII.D.1). Tests of population expansion were both negative, but only Fu's Fs statistic was significant (D=-0.65, p=0.29, Fs=-7.19, p<0.0001).

Phylogeographic Analyses

The most suitable model for Bayesian analysis of the relationships among *X. virginica* and *X. micans* haplotypes was HKY+G (γ shape=0.186). There was very little resolution in either species, and the majority of haplotypes was polytomic in both, suggesting that the phylogenetic signal among these samples was low (Fig. VII.D.3). There were four clades recovered in *X. virginica*, and all showed some geographic affinity. The well-supported XV4+ XV20 clade was exclusive to the west (blue, Fig. VII.D.3, top), yet the other western clade (XV12+XV15+XV22+XV37; light purple, Fig. VII.D.3, top) had low support and included a sample from Alabama. The larger eastern clade included eight haplotypes, including the common

haplotypes XV1 and XV17 (orange, Fig. VII.D.3, top), along with a subclade (XV26+XV27; red, Fig. VII.D.3, top) exclusive to Florida. In the analysis of *X. micans* haplotypes, four clades were recovered, with less geographic association. The XM8+XM10 clade was exclusive to the west and contained the common haplotype XM8 (blue, Fig. VII.D.3, bottom). The eastern XM25+XM29+XM33 clade was exclusive to this region, but had low support (orange, Fig. VII.D.3, bottom). Two additional clades, XM5+XM22 (light purple, Fig. VII.D.3, bottom) and XM11+XM18 (red, Fig. VII.D.3, bottom), consisted of samples from with mixed east-west origins.

Because these data were not tree-like, network models were additionally used to explore relationships among haplotypes within each species. The minimum spanning network trees for *X. virginica* and *X. micans* are shown in Fig. VII.D.4 and Fig. VII.D.5, respectively. Haplotype XV6 was by far the most common haplotype encountered in *X. virginica*, accounting for 41% of all samples. Its basal positon in network trees with abundant multifurcations (Figs. VII.D.4 and VII.D.6) and widespread distribution (Fig. VII.D.1) also give evidence for XV6 as the ancestral haplotype (Posada and Crandall 2001) within *X. virginica*. Haplotype XV1 is a candidate secondary ancestral haplotype, as it is connected to six other haplotypes and was relatively common and widespread (Fig. VII.D.1). In the splits diagram, XV1 is the basal member of a group separated by two relatively long splits [XV1+XV26+XV27+XV31+XV32+XV36] (Fig. VII.D.6). This group is primarily eastern and is a portion of the eastern clade presented in the Bayesian tree (orange, Fig. VII.D.3). The remainder of that eastern clade stems from XV17 in both the minimum spanning tree (Fig. VII.D.4) and the neighbor net diagram [XV17+XV28+XV34+XV28] (Fig. VII.D.6). It, like XV1, gives rise to multiple connections in

both networks (n=4) and was relatively common and widespread in this sample (Fig. VII.D.1). Together, this suggests a secondary ancestral position for XV17 as well.

Both *X. micans* networks suggest that the basal haplotype of this species is XM4 (Figs. VII.D.5, VII.D.7). The remaining relationships are more complex than in the case of *X. virginica*, however. The eastern group represented in orange in both the Bayesian tree (Fig. VII.D.3) and the minimum spanning tree (Fig. VII.D.5) was not separable in the splits diagram (Fig. VII.C.7). The overall starburst pattern of relationships in both networks suggests a close relationship among haplotypes indicative of recent expansion with little subsequent diversification.

Xylocopa virginica showed isolation by distance for the overall dataset (r=0.23, p=0.017) and for eastern populations (r=0.33, p=0.027), but no such signal was evident in western populations (r=-0.03, p=0.56; Fig. VII.D.8). None of the *X. micans* populations had significant isolation by distance (overall: r=-0.107, p=0.76; eastern: r=-0.33, p=0.83; western: r=0.085, p=0.38; Fig. VII.D.9).

Distribution of pairwise differences (mismatch distribution) among *X. virginica* samples suggested that these populations might have recently undergone demographic expansion (τ=1.5, Fig.VII.D.10). The shape of the curve was unimodal, Harpending's raggedness index was low and insignificant, and a population expansion hypothesis could not be rejected with these data (*Hri*=0.06, p=0.08). Results of mismatch distribution analysis of *X. micans* were similar and also support a model of recent demographic expansion (τ=2.18, *Hri*=0.06, p=0.17, Fig.VII.D.11).

Analysis of population structure with AMOVA confirmed that some differentiation was evident among populations of both species. In both *X. virginica* and *X. micans*, the majority of genetic divergence occurred within populations (58% and 98%, respectively), rather than among

populations (24% and ~0%, respectively) or between groups east and west of the Mississippi River (18% and 4%, respectively, Table VII.D.2). Fixation index estimates were significant, however, with overall Φ_{ST} = 0.197 and 0.032, respectively (both $P < 0.05$). Both species showed significant differentiation between eastern and western populations, but this was more pronounced in *X. virginica* (Φ_{CT} = 0.180 and 0.047, respectively; both $P < 0.05$). *Xylocopa virginica* additionally showed substantial differentiation among subpopulations within regions (Φ_{SC} = 0.291, $P < 0.0005$).

The eastern population of *X. virginica* was more diverse than the western one (East: H_d=0.81±0.01; West: H_d=0.59±0.05), yet the opposite was true for *X. micans* (East: H_d=0.94±0.03; West: H_d=0.86±0.05; Table VII.D.3). Examination of pairwise Φ_{ST} values in *X. virginica* shows that the intraregional heterogeneity is much stronger among eastern populations (average Φ_{ST} =0.32±0.30 SD) than western ones (average Φ_{ST} =0.07±0.12 SD; Fig. VII.D.12). In particular, five populations seem unaligned with the remaining eastern populations: Gibson, Tipton and Weakley Counties in Tennessee, Baltimore County, Maryland and Ontario, Canada. Baltimore is the most geographically eastern and, because the haplotype XV18 only occurred in this population, the haplotype composition of this population is unlike any other. The remaining populations from Tennessee and Canada have pairwise Φ_{ST}=0, reflecting the dominance of XV1 in these populations. Population differentiation is far lower among pairwise comparisons of *X. micans* populations, but the trend is the opposite (Fig. VII.D.13). Eastern populations of *X. micans* had pairwise Φ_{ST}=0, but western populations had mean pairwise Φ_{ST}=0.23±0.28 SD. With so few populations (n=8), it is difficult to identify geographic trends, but high fixation values in pairs with Jefferson Davis Parrish, Louisiana (Φ_{ST}=0.34±0.20 SD) reflect the presence of haplotype XM8, which only occurred in this population and in Harris County, Texas.

E. DISCUSSION

From analyses of museum records and recent collection data, it is apparent that *X. micans* has expanded its range into areas north of its historical range. Hurd's (1955) examination of records between 1888 and 1955 restricted the range of *X. micans* to the southeastern US along the Atlantic coast (Fig. VII.B.1). Although the single 1888 record from Illinois is not likely to represent an established population, the 1955 record from Bolivar County, Mississippi suggests that *X. micans* could have been present in the expansion area since that time. Specimens of *X. micans* were collected in multiple locations in Arkansas between 2006 and 2011 (Warriner 2010; Tripodi and Szalanski 2011). A single specimen was also collected in western Tennessee in 2011 (Haywood County, this study) and 14 were collected in northern Mississippi in 2013 (Lafayette County, this study). These new records occurred 150 to 350 km from the expected distribution of *X. micans* based on historical occurrences (Fig. VII.B.1). Although past anthropogenic introductions of *Xylocopa* are known (Hurd and Moure 1963), multiple occurrences of *X. micans* over time suggest that *X. micans* is a resident of the region. Also, the genetic diversity of *X. micans* in the expansion region was high (H_d=0.95±0.05) and comparable to that of the known range (H_d=0.91±0.03), unlike what is expected under most introduction scenarios (but see Johnson and Starks 2004 for an interesting exception). The presence of unique haplotypes in the expansion range (XM2: Prairie County, Arkansas and XM7: Little River County, Arkansas) is noteworthy, but with such little sampling from the known range, it is also likely that these haplotypes are present elsewhere in the range of *X. micans.* Further studies of both museum records and genetic diversity are warranted to determine the nature of this expansion.

Both *X. virginica* and *X. micans* show signals of populations that have recently undergone demographic expansion (mismatch distributions: Figs. VII.D.10 and VII.D.11; *D* and

Fs statistics: Table VII.D.1). This is unsurprising under any of the proposed hypotheses, since all three suggest that recolonization largely took place ~12,000 years ago. A comparison of the population differentiation evidence for both species suggests that they did not recolonize in the same fashion, however. *Xylocopa virginica* exhibited substantial isolation by distance, particularly in the larger, eastern portion of its range (Fig. VII.D.8), and *X. micans* exhibited no such signal (Fig. VII.D.9). Populations of *X. virginica* were not only more highly differentiated from western populations than *X. micans*, but also showed greater differentiation among populations (Table VII.D.2), with most of the variation evident in the east (Fig. VII.D.12). Although sampling was lower for *X. micans*, both haplotype number and diversity were high in *X. micans* (Table VII.D.1), suggesting that these measures were sampled adequately. *Xylocopa micans* appears to have a panmictic distribution with little evidence for population differentiation consistent with the single-refugium hypothesis. Although the location of this refugium is unknown, there is no evidence to refute the Central-American-refugium hypothesis for this species. On the other hand, *X. virginica* shows evidence in support of the hypothesis of multiple refugia. Populations to the west of the Mississippi River are largely similar, suggesting a single refugium west of the river. Eastern populations are quite different from western populations and more variable, suggesting that more than one additional refugium could have persisted in the east. This is consistent with the morphology exhibited by this polymorphic species, which shows two main subtypes in the east and one in the west (Hurd and Moure 1963).

The *X. virginica* minimum spanning network suggests that haplotypes XV1 and XV17 are additional basal groups that could indicate potential sources of diversity radiating from eastern refugia (Fig.VII.D.5). Haplotype XV17 was particularly common in samples from Florida, and XV1 was very common in populations just east of the Mississippi River (Fig.

VII.D.1). Although Floridian LGM refugia are well known (reviewed in Howden 1969; Swenson and Howard 2005), the possibility of a Lower Mississippi Alluvial Plain refugium for *X. virginica* is worth noting. Temperatures at the LGM in this region have been estimated to range in mid-January as low as -16°C to mid-July highs of 14°C, although temperatures might have only been about 5–10°C cooler in the area (Jackson, *et al.* 2000). Because contemporary populations of *X. virginica* are found in regions with mean winter temperatures as low as -14°C (Skandalis, *et al.* 2011), it is not unreasonable to assume that some areas of the Lower Mississippi Alluvial Plain could have been habitable. Additionally, the plant community in the region during this period included potential food plants, such as tulip tree (*Liriodendron tulipifera*), willows (*Salix* spp.) and members of the Asteraceae, as well as softwoods that could be used for nesting, such as spruces (*Picea* spp.) (Royall, *et al.* 1991; Jackson, *et al.* 2000). Samples from the Ontario, Canada site represent the only specimens analyzed from areas that were actually glaciated during the LGM; all others were south of the glacier's extent. All 12 samples from this site had haplotype XV1, and no other site with more than 10 samples showed such low haplotypic diversity. Haplotype XV1 was also common in the Pennsylvania site closest to Ontario, suggesting that it is common in the area. These data offer intriguing insights into the population structure of *X. virginica*, but discovering the locations of specific refugia will require finer-scale sampling and analysis.

The data presented here provide support for the application of Hewitt's (2000) initial-colonizer hypothesis to the question of why these two species exhibit such different range sizes. *Xylocopa virginica* has occupied the greater portion of eastern North America, while *X. micans*, though expanding, has remained relatively restricted to its southern extremes. *Xylocopa virginica* most likely persisted through the last LGM in multiple refugia, including at least one location

east of the Mississippi River. This would have given *X. virginica* a head start as the glaciers receded and both species began recolonizing areas as they became suitable. The fact that both species exhibited clear signals of population expansion disagrees with the predictions of the initial-colonizer hypothesis, yet the genetic diversity of *X. micans* sampled in this work is impressive and clearly meets the expectations of the initial-colonizer hypothesis. Haplotypic diversity reached near unity (H_d=0.91), with 69% of haplotypes only present as singletons (Table VII.D.1). On the whole, the haplotypic diversity of *X. virginica* was also high (H_d=0.78), but all four populations from the northeast exhibited haplotypic diversity lower than the eastern average (Table VII.D.3; Ontario, Canada: H_d=0, Centre County, Pennsylvania H_d=0.75, Baltimore County, Maryland: H_d=0.27, Mecklenburg County, Virginia: H_d=0.60), as would be expected in populations derived from the leading edge of an expansion.

In summary, the evidence presented here lends support to the following conclusions: 1) *X. micans* has expanded its range north of its historical bounds, 2) *X. micans* recolonized North America from a single refugium, 3) *X. virginica* has recolonized North America from multiple refugia and 4) the presence of *X. virginica* ostensibly blocked *X. micans* from occupying a larger portion of North America. Range size disparity is likely caused by a combination of evolutionary, ecological and physiological factors (Calosi, *et al.* 2010). Only the first of these factors has been addressed here, and ecological and physiological aspects remain unexplored. These findings suggest that this system might be ideal for comparative analyses, particularly those involving species' responses to climatic changes. There is a rich literature on *Xylocopa* physiology and temperature tolerances, particularly in *X. virginica*, but none on *X. micans* (*e.g.* Chappell 1982; Baird 1986; Watmough and Vanark 1989; Skandalis, *et al.* 2011). The two species are ecologically similar, but likely exhibit differences in flower and nest-site preferences.

Because they co-occur in part of their range, direct analyses of ecological niche and competition could be easily conducted.

F. Acknowledgments

I wish to thank the curators Edward Riley (TAMU), James Wiley (FSCA), Jeffery Barnes (UAAM) for access to the specimens used in this work. The following institutions granted collecting permits: Arkansas Natural Heritage Association (permit numbers S-NHCC-12-013 and S-NHCC-13-010), Florida Fish and Wildlife Conservation Commission, Southwest Regional Office (Permit # SUO-49182), Louisiana Department of Wildlife and Fisheries (Permit # LNHP-13-048), Mississippi Museum of Natural Science, Mississippi Department of Wildlife, Fisheries and Parks, Oklahoma Department of Wildlife (Permit #5605) and Southwestern Florida Water Management District (Permit# 10-200). I am particularly grateful to Clinton Trammel for collecting assistance throughout the southeast and to Manny Lopez and Paul Elliot for assistance in finding specimens in Florida. Lastly, I would like to thank the following individuals for providing additional specimens: Adam Alford, Amber Andrews, Cathy Callaway, Chris Carlton, Kelly Carruthers, Lynda Conlan, Travis Edwards, Jake Farnum, Briana Godby, Johnathan Gossage, W.D. Grissom, Jack Grubaugh, Dawn Hall, Sasha Kay, Soo-Hoon Kim, Norm Lavers, Joy Layton, Anna Lewis, Barbara Lewis, Shawn Martin, Bill Miller, Pat Miller, Dee Ripko, Eugene Scarpulla, Xime Stewart, Hannah Tripodi, Lloyd Tripodi, Jess Vickruck and Cliff Westbrook.

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Table VII.D.1. Estimated genetic diversity and demographic parameters for the complete and reduced datasets of *X. virginica* and the complete and expansion datasets of *X. micans*.

Dataset	N	Pops	*H*	*S*	H_d	π	*D*	*Fs*
X. virginica								
Complete	382	55	38	35	0.78 ± 0.02	1.36 ± 0.84	-2.04***	-28.11***
Reduced	304	52	35	32	0.78 ± 0.02	1.41 ± 0.87	-1.99**	-28.02***
X. micans								
Complete	73	14	35	29	0.91 ± 0.03	2.11 ± 1.19	-2.04**	-27.22***
Expansion	14	5	10	7	0.95 ± 0.05	1.18 ± 1.11	-0.65	-7.19***

N=sample size, Pops=number of sampled populations (counties), *S*=number of polymorphic nucleotide sites, H_d: haplotypic diversity±SD, π: mean number of pairwise differences ±SD, *D:* Tajima's *D* statistic, *Fs*: Fu's F statistic. All measures of uncertainty are standard deviations. Significance for *D* and *Fs* determined with 10,000 simulations: **P*<0.05, ** *P* < 0.01, ****P*<0.001

Table VII.D.2. AMOVA estimates and hypothesis testing of *a priori* East-West population differentiation.

	X. virginica			*X. micans*		
Source of variation (Φ-Statistic)	% Variation	Fixation Index	*P*-value	% Variation	Fixation Index	*P*-value
Between East and West (Φ_{CT})	18.0	0.180	< 0.0005	3.94	0.039	0.029
Among subpopulations in regions (Φ_{SC})	23.8	0.291	< 0.0005	-2.09	-0.022	0.77
Within subpopulations (Φ_{ST})	58.1	0.419	< 0.0005	98.2	0.019	0.42
Overall (Φ_{ST})	-	0.197	< 0.0005	-	0.032	0.013

Negative values of fixation indices should be interpreted as zeroes. Significance determined with 16,000 permutations.

Table VII.D.3. Estimated genetic diversity and demographic parameters for *X. virginica* and *X. micans* by population group.

Species	Group	H_d	π	*D*	*Fs*
X. virginica	East	0.81 ± 0.01	1.48 ± 0.90	-1.47*	-15.65***
	West	0.59 ± 0.05	0.933 ± 0.65	-1.96**	-17.387***
	All	0.78 ± 0.02	1.41 ± 0.87	-1.99***	-28.02***
X. micans	East	0.94 ± 0.03	2.26 ± 1.3	-1.20	-9.04***
	West	0.86 ± 0.05	1.82 ± 1.1	-2.12 *	-21.93***
	All	0.91 ± 0.03	2.11 ± 1.2	-2.04***	-27.22***

H_d: haplotypic diversity±SD, π: mean number of pairwise differences±SD, *D:* Tajima's *D* statistic, *Fs*: Fu's F statistic. All measures of uncertainty are standard deviations. Significance for *D* and *Fs* determined with 10,000 simulations: **P*<0.05, ***P*<0.01, ****P*<0.001.

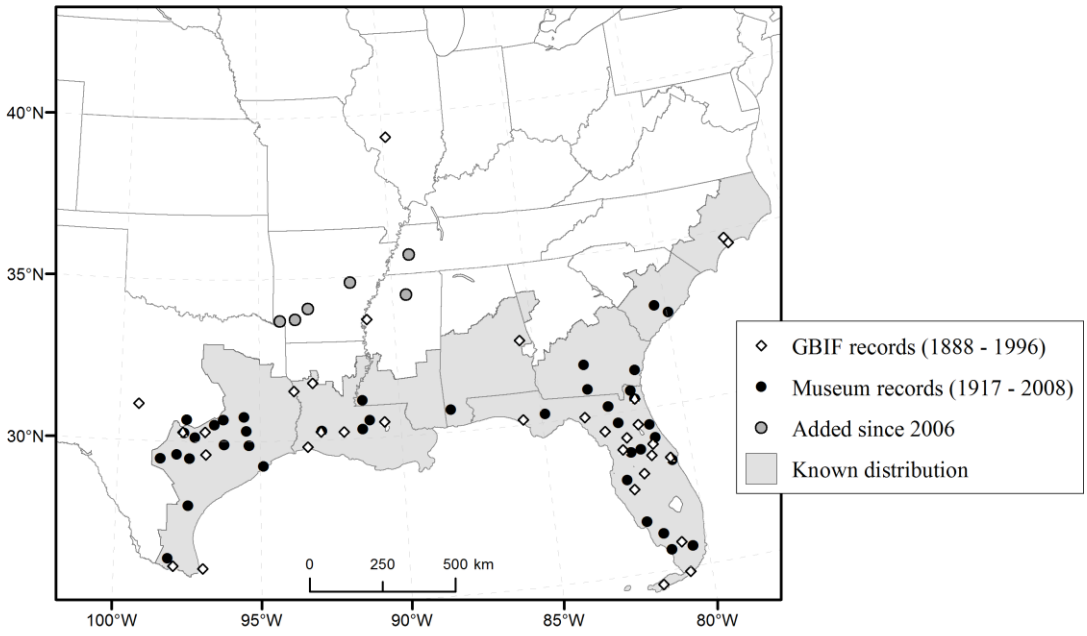

Figure VII.B.1. The distribution of *X. micans* in the southern United States with recent records outside of the known range. Shaded area indicates the known range as described by Hurd (1955). White diamonds: records obtained from the GBIF database; black dots: museum specimens morphologically verified in this work; grey dots: records outside of this range published since 2006 (Warriner, 2010; Tripodi and Szalanski, 2011) plus records from northern Mississippi and Tennessee in this work.

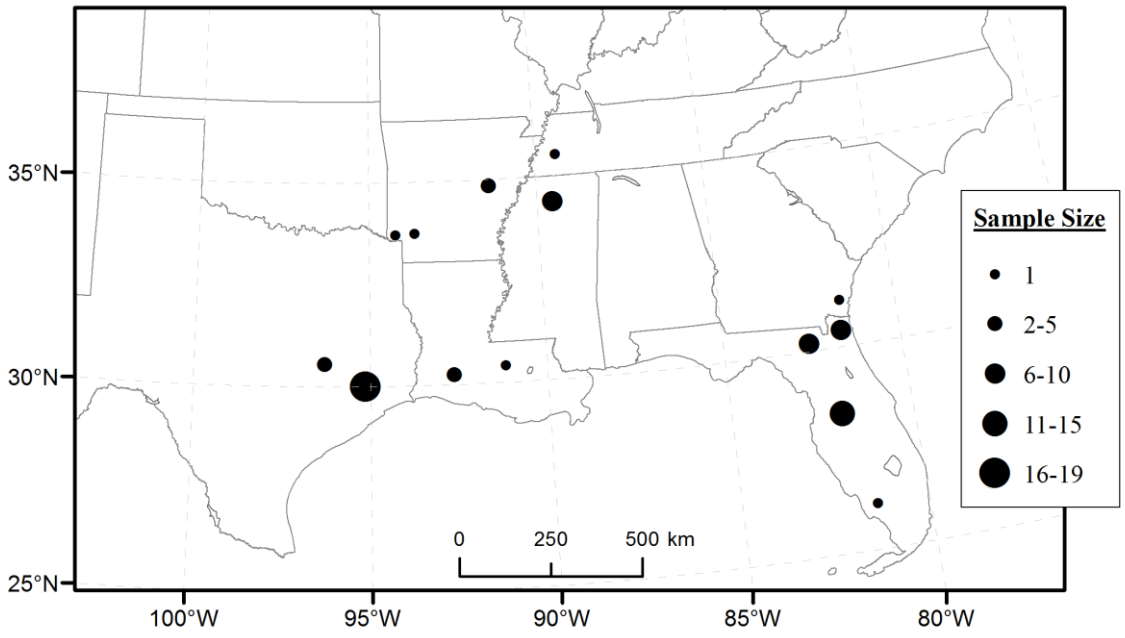

Figure VII.C.1. Sample locations and sample sizes of *Xylocopa micans* used for genetic analysis.

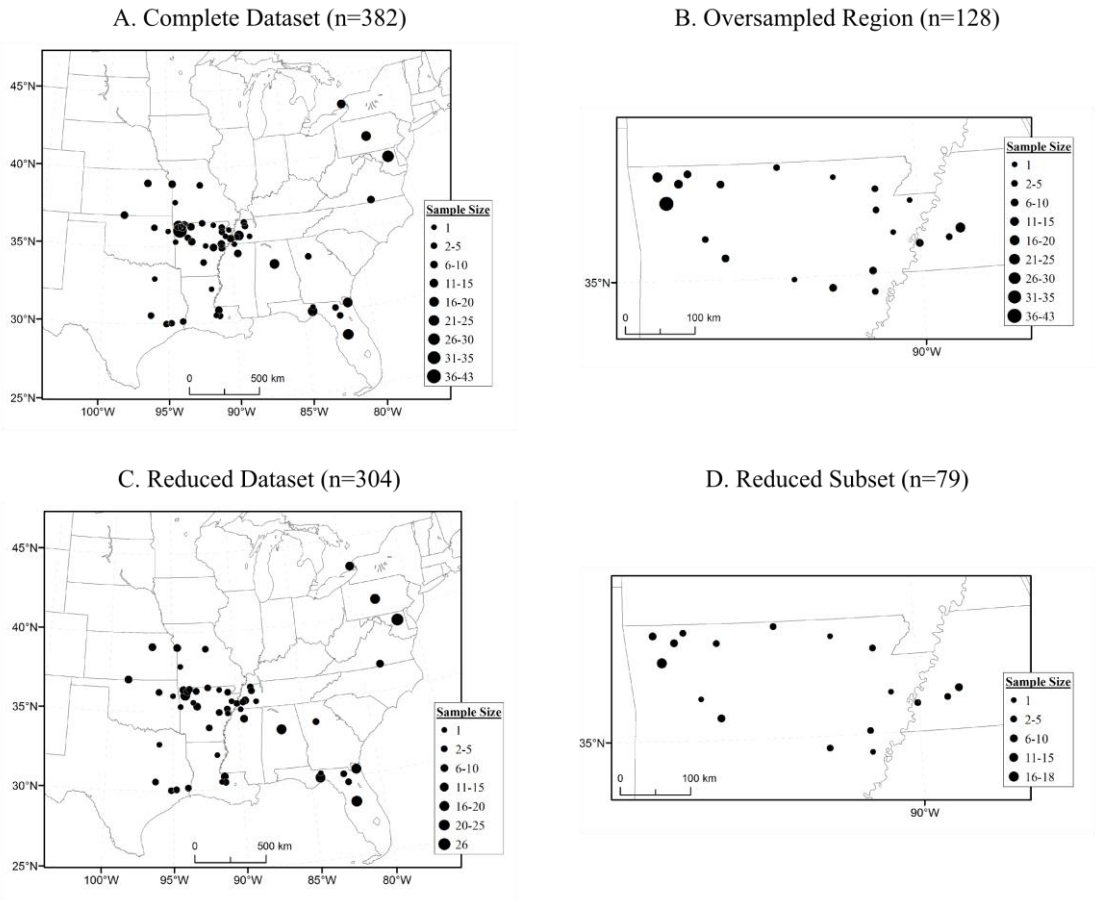

Figure VII.C.2.A–D. Sample locations and sample sizes of *Xylocopa virginica* used for genetic analysis. A) all samples, B) detail of the oversampled region, C) reduced sample set used for analysis and D) detail of the reduced subset of data in the oversampled region.

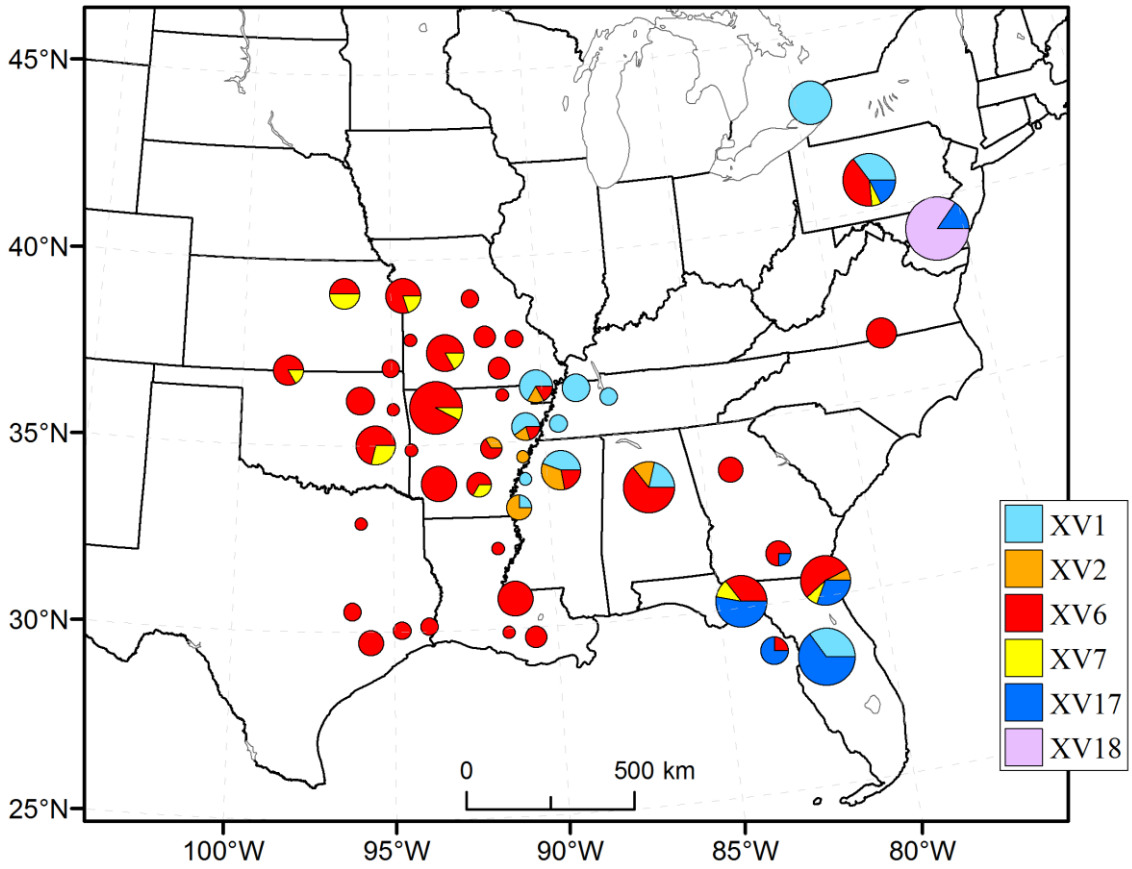

Figure VII.D.1. The distribution and abundance of the six most common haplotypes of *X. virginica*. Colors indicate haplotypes (light blue=XV1 (n=49), orange=XV2 (n=13), red=XV6 (n=125), yellow=XV7 (n=13), blue=XV17 (n=35), light purple=XV18 (n=22)); pie slices represent the proportion of samples at each location that had each haplotype; size of the pie is relative to total sample number at each location; locations are approximate to prevent overlap among charts.

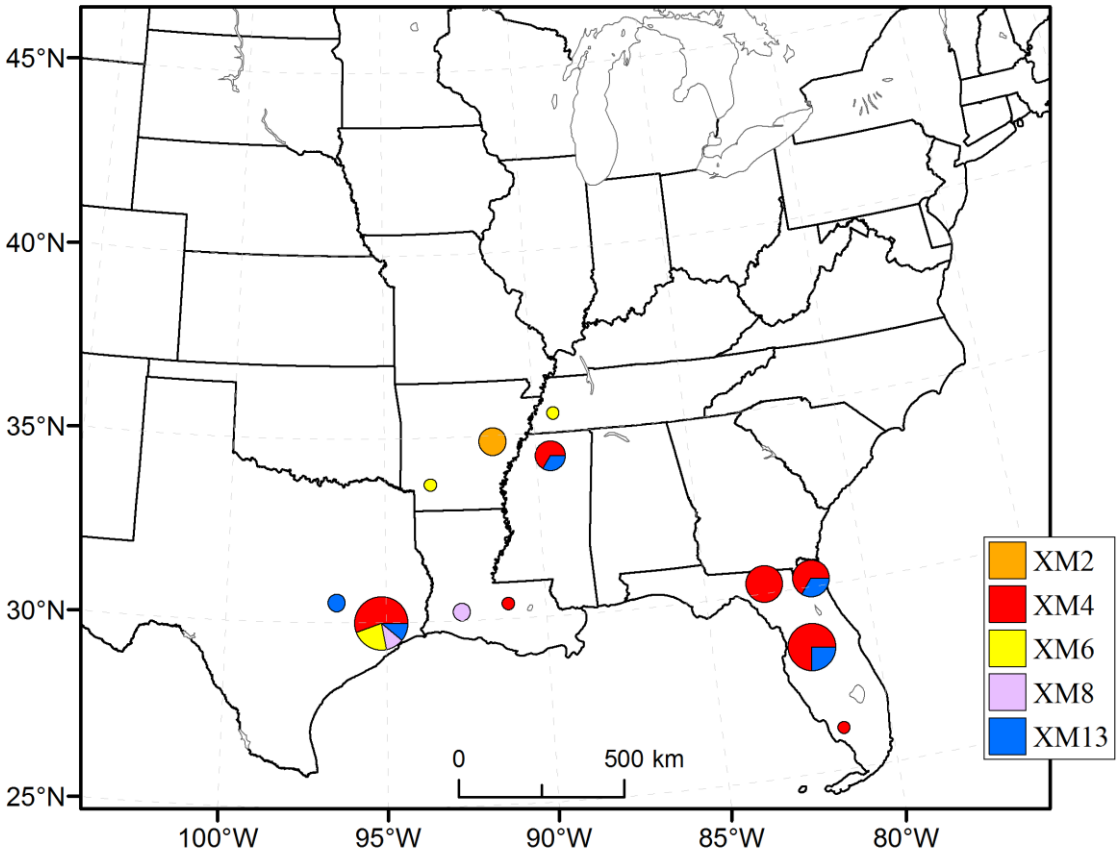

Figure VII.D.2. Distribution and abundance of the five most common haplotypes of *X. micans*. Colors indicate haplotypes (orange=XM2 (n=3), red=XM4 (n=21), yellow=XM6 (n=4), light purple=XM8 (n=3), blue=XM13 (n=6)); pie slices represent the proportion of samples at each location that had each haplotype; size of the pie is relative to total sample number at each location; locations are approximate to prevent overlap among charts.

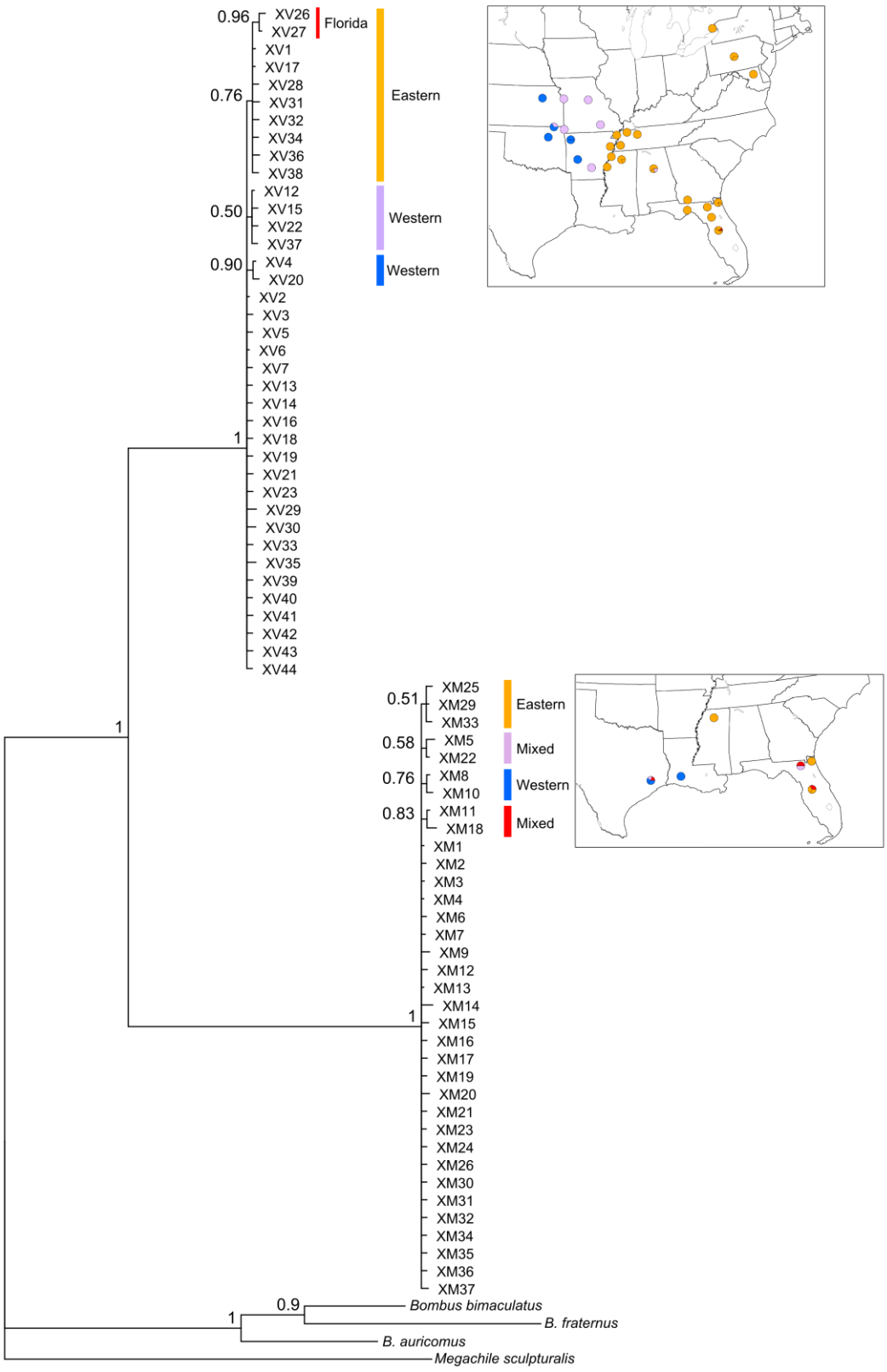
0.96
XV26
XV27
Florida
XV1
XV17
XV28
0.76
XV31
XV32
XV34
XV36
XV38
Eastern
XV12
0.50
XV15
XV22
XV37
Western
0.90
XV4
XV20
Western
XV2
XV3
XV5
XV6
XV7
XV13
XV14
XV16
1
XV18
XV19
XV21
XV23
XV29
XV30
XV33
XV35
XV39
XV40
XV41
XV42
XV43
XV44
1
0.51
XM25
XM29
XM33
Eastern
0.58
XM5
XM22
Mixed
0.76
XM8
XM10
Western
0.83
XM11
XM18
Mixed
XM1
XM2
XM3
XM4
XM6
XM7
XM9
XM12
XM13
XM14
1
XM15
XM16
XM17
XM19
XM20
XM21
XM23
XM24
XM26
XM30
XM31
XM32
XM34
XM35
XM36
XM37
0.9
Bombus bimaculatus
1
B. fraternus
B. auricomus
Megachile sculpturalis

Figure VII.D.3. (Preceding page) Bayesian phylogenetic tree of a 658 bp region of *COI* for *X. virginica* and *X. micans* haplotypes. Haplotypes beginning with *XV* are those of *X. virginica;* those with the *XM* prefix are *X. micans.* Numbers at nodes are posterior probabilities. Branch lengths are scaled by the number of nucleotide substitutions per site, as shown in the scale bar. The geographic origins of recovered clades are color coded and noted in the inset maps to the right of the tree for each species (top: *X. virginica;* bottom: *X. micans*).

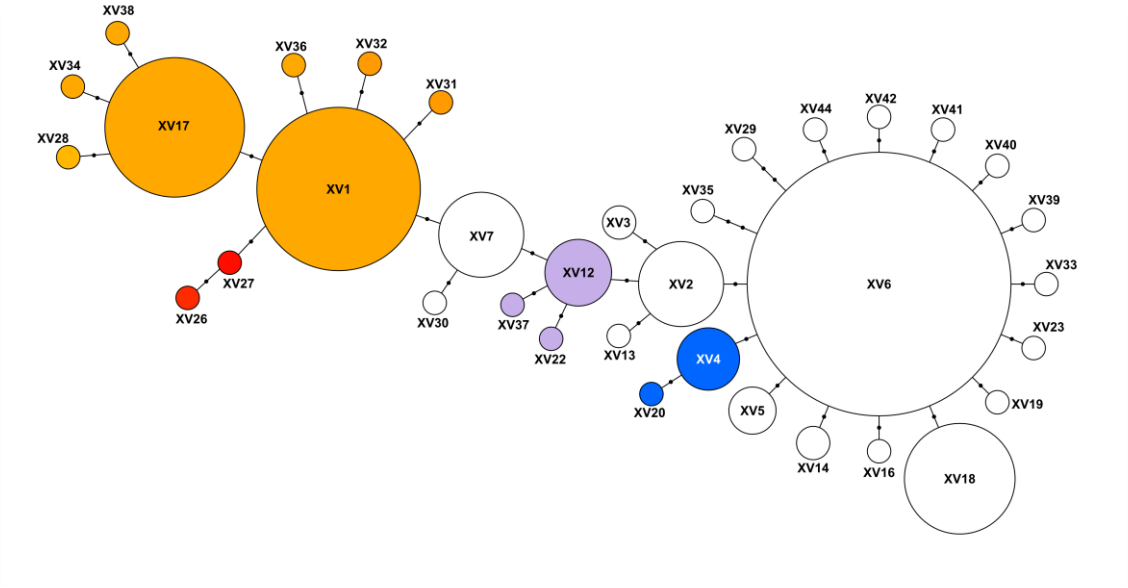

Figure VII.D.4. Minimum spanning network tree of 35 *X. virginica* haplotypes of a 658 bp region of *COI*. The circles represent haplotypes analyzed in this study, and are sized relative to the number of individuals with that haplotype. The number of dots along a line represents the number of mutational step between haplotypes. Sample size for each haplotype ranged from one to 125 (XV6), and colors correspond to the clades recovered in the Bayesian tree (Fig. VII.C.1).

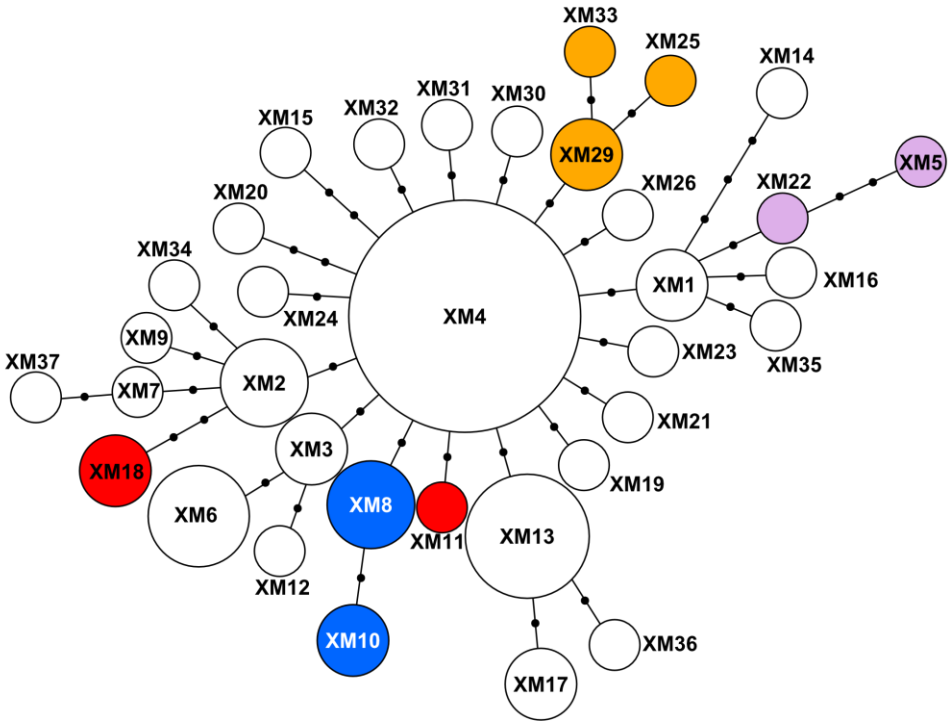

Figure VII.D.5. Minimum spanning network tree of 35 *X. micans* haplotypes of a 658 bp region of *COI*. The circles represent haplotypes recovered in this study, and are sized relative to the number of individuals with that haplotype. The number of dots along a line represents the number of mutational step between haplotypes. Sample size for each haplotype ranged from one to 21 (XV4), and the colors match the clades recovered in the Bayesian tree (Fig. VII.C.1).

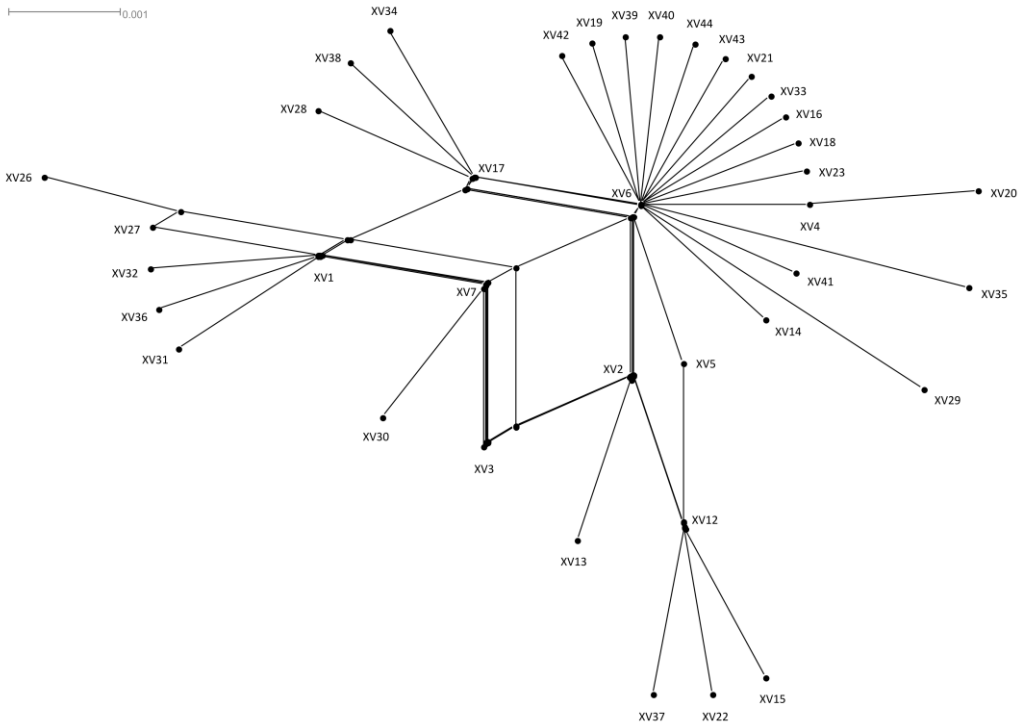

Figure VII.D.6. Neighbor net splits diagram illustrating uncertainty in relationships among *X. virginica* haplotypes. Each haplotype is a node, and the lines connecting nodes are scaled as weighted, uncorrected p-distances between haplotype groups (note scale bar).

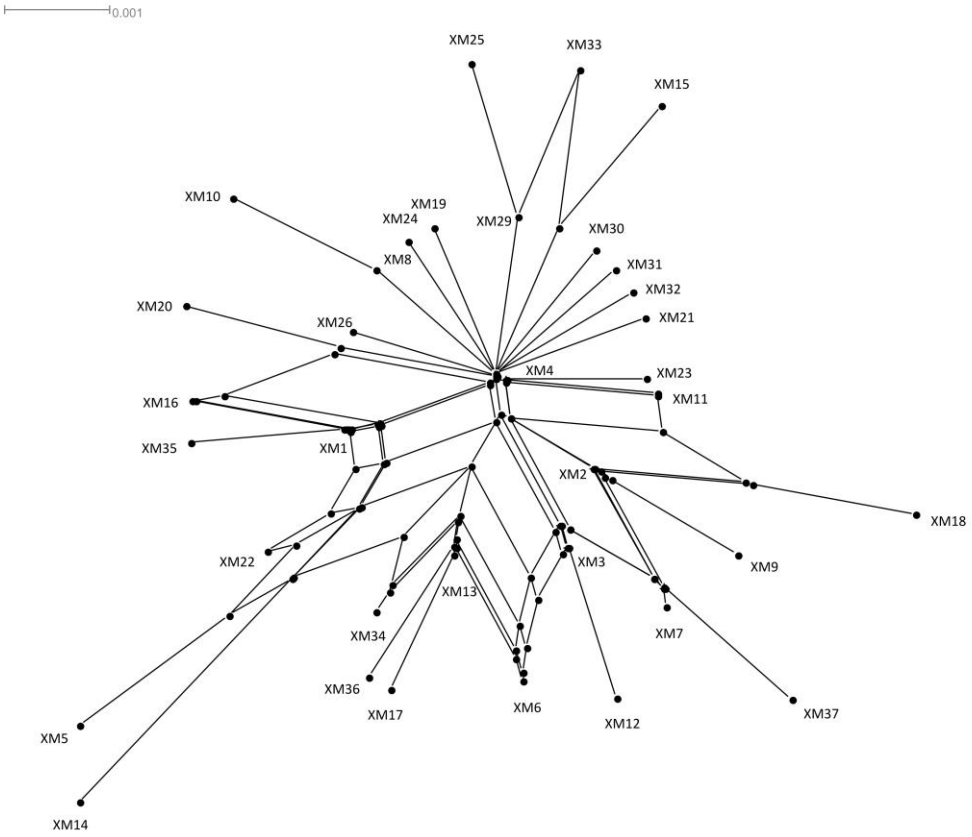

Figure VII.D.7. Neighbor net splits diagram illustrating uncertainty in relationships among *X. micans* haplotypes. Each haplotype is a node, and the lines connecting nodes are scaled as weighted, uncorrected p-distances between haplotype groups (note scale bar).

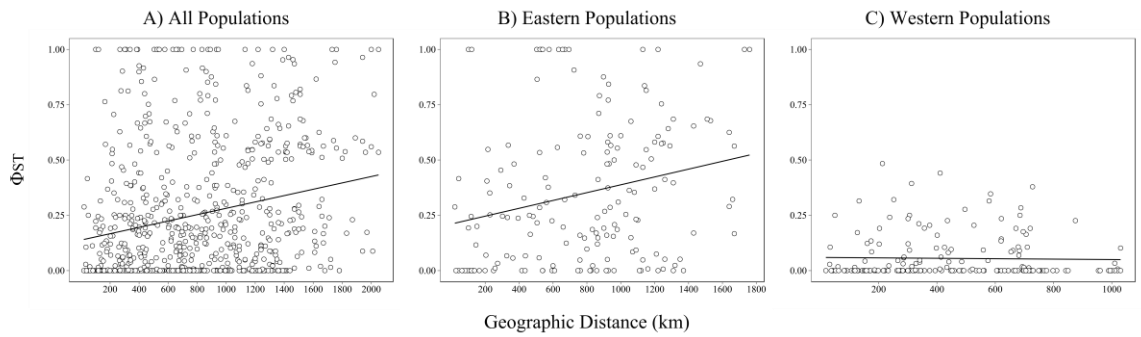

Figure VII.D.8. Pairwise isolation (*ΦST*) by distance (km) among populations of *X. virginica.* Results of the Mantel tests were A) all populations: r = 0.23, p= 0.017, B) eastern populations: r = 0.24, p= 0.027, C) western populations: r = -0.03, p = 0.56.

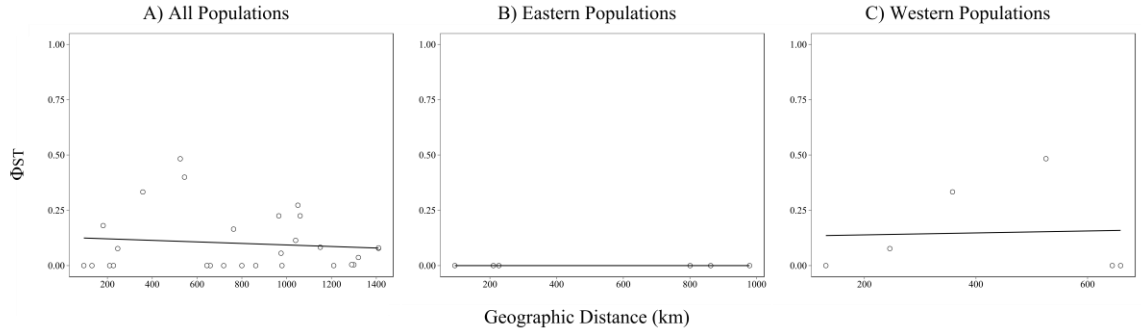

Figure VII.D.9. Pairwise isolation (*ΦST*) by distance (km) among populations of *X. micans.* Results of the Mantel tests were A) all populations: r = -0.107, p= 0.76, B) eastern populations: r = -0.33, p= 0.83, C) western populations: r = 0.085, p = 0.38.

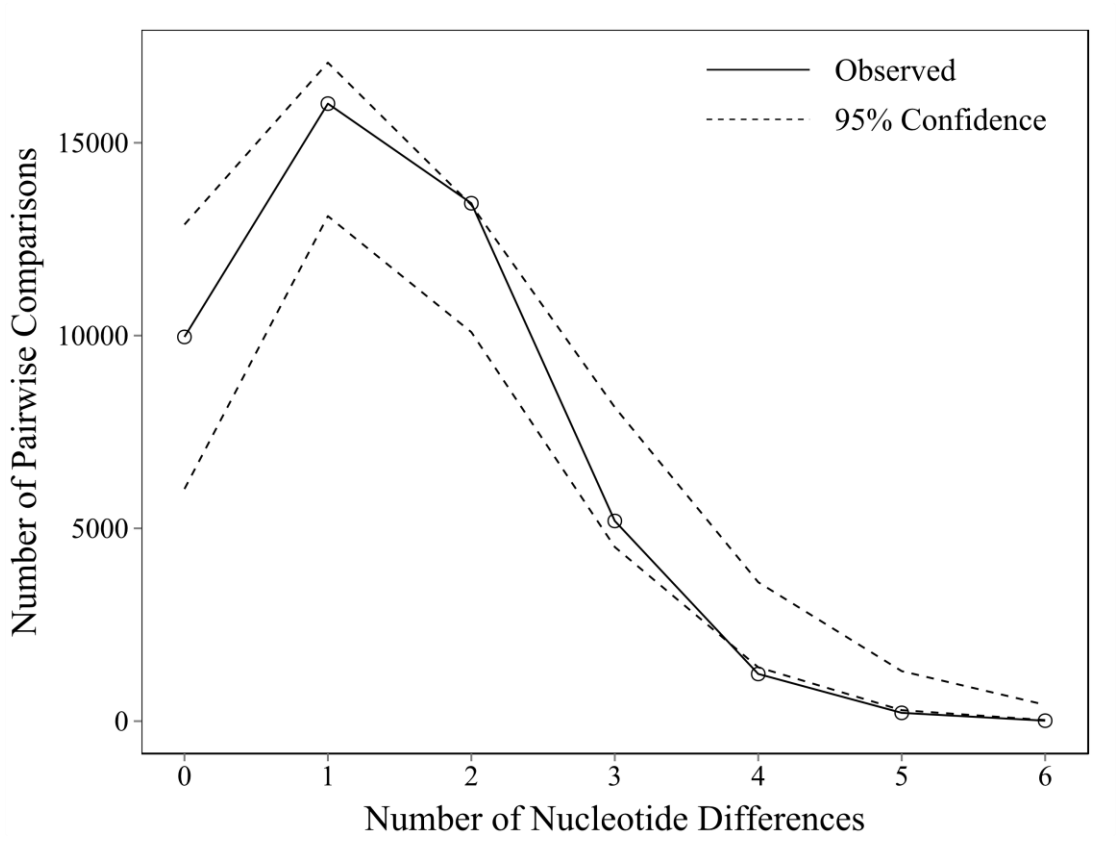

Figure VII.D.10. Distribution of pairwise nucleotide differences (mismatch distribution) among *X. virginica* samples illustrating that these populations might have recently undergone demographic expansion. τ=1.5, *Hri*=0.06, *p*=0.08. Solid line: observed, dotted line: 95% confidence intervals around model expectations, based on 10,000 bootstrap replications.

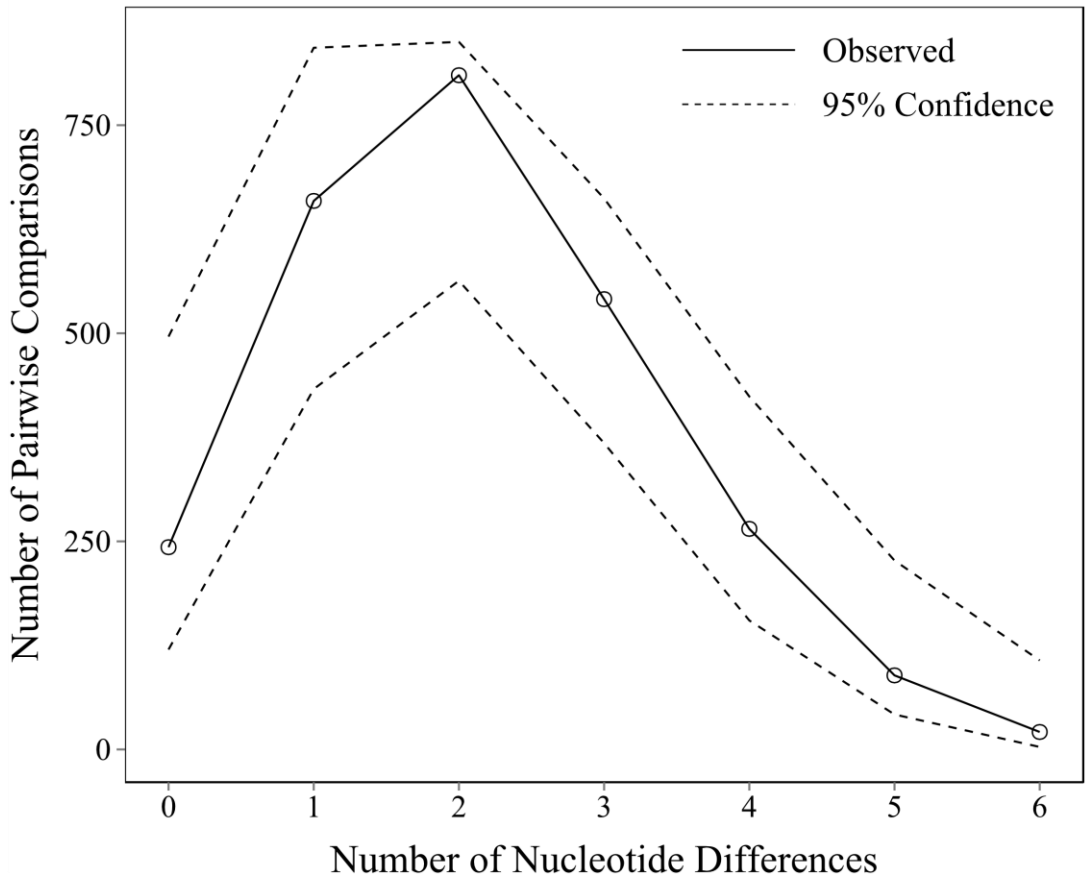

Figure VII.D.11. Mismatch distribution among *X. micans* samples illustrating that these populations might have recently undergone demographic expansion. τ=2.18, *Hri*=0.06, p=0.17. Solid line: observed, dotted line: 95% confidence intervals around model expectations, based on 10,000 bootstrap replications.

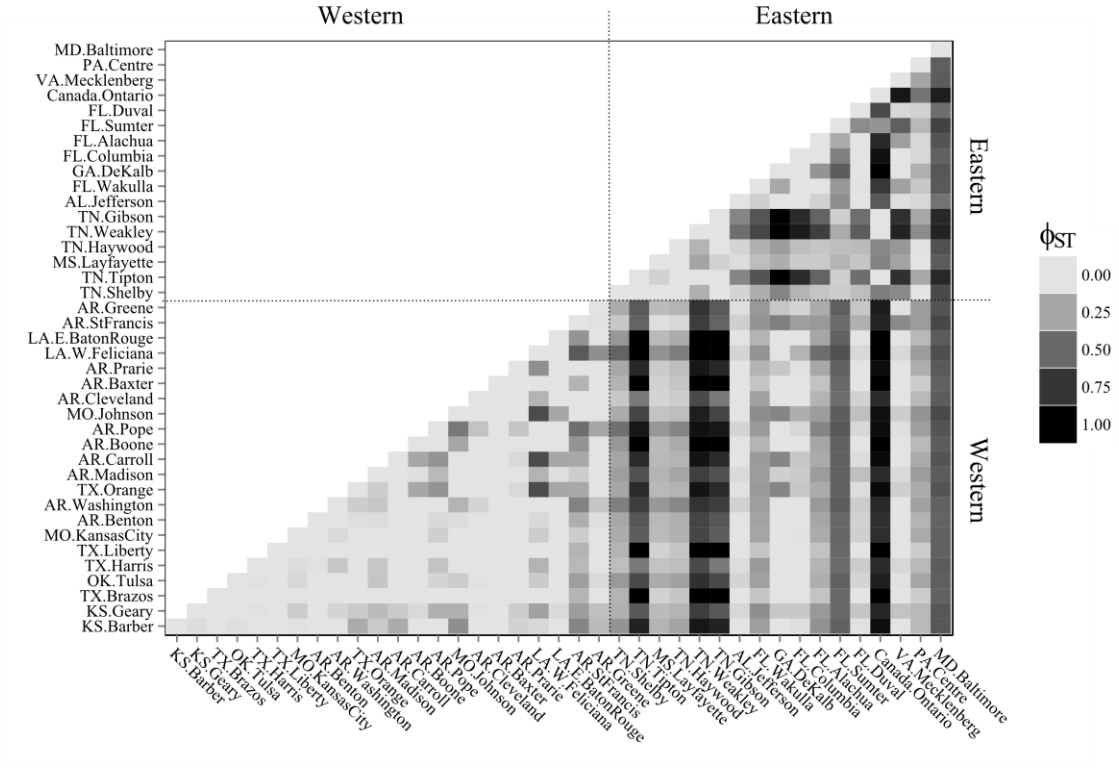

Figure VII.D.12. Pairwise *ΦST* values between populations of *X. virginica* with samples larger than one. Populations are arranged by longitude from west to east, and populations are named with two-letter codes for states, followed by the county name. Dotted lines indicate *a priori* designations of eastern and western populations. Values of *ΦST* range from 0 (light grey) to 1 (black).

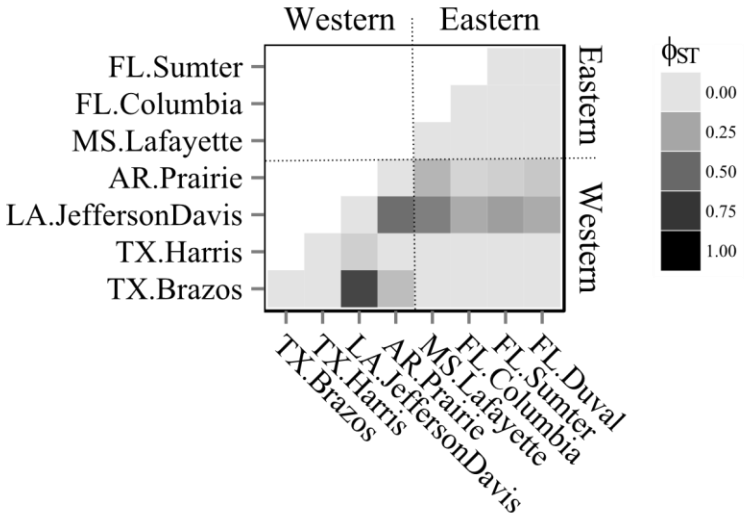

Figure VII.D.13. Pairwise *ΦST* values between populations of *X. micans* with samples larger than one. Populations are arranged by longitude from west to east, and populations are named with two-letter codes for states, followed by the county name. Dotted lines indicate *a priori* designations of eastern and western populations. Values of *ΦST* range from 0 (light grey) to 1 (black).

H. APPENDIX VII.I. HOW TO INTERPRET SPLITS NETWORK DIAGRAMS.

Splits networks offer a powerful tool to visually capture phylogenetic relationships among taxa that do not follow the typical topography of a bifurcating tree, such as trees with multiple polytomies and unresolved branches. The following diagrams illustrate how to interpret splits network diagrams using the *X. virginica* haplotypes found in this study (Fig. VII.H.1). Each split is represented by a set of parallel lines (coded red in the following diagrams). These are analogous to the branches on a phylogenetic tree, in that the length corresponds to phyletic distance and that removal of any split would result in creating two separate trees. The nodes in a splits network are either labeled as haplotypes or unlabeled. The unlabeled nodes represent connections, but unlike similar nodes in minimum spanning networks, they are not analogous to unrepresented ancestral haplotypes. A set of nodes is joined as a group by the split(s) that they have in common. The length of the split increases with the difference between the groups it separates. In the following diagrams 1–7, the set of parallel lines representing a single split is highlighted red. The red polygon contains all of the members of one of the two groups on either side of the split. Each node can be a member of more than one group, however. The last diagram (8) shows the two (of four) clades also recovered by Bayesian phylogenetic analysis, color coded to correspond with Fig. VII.D.3.

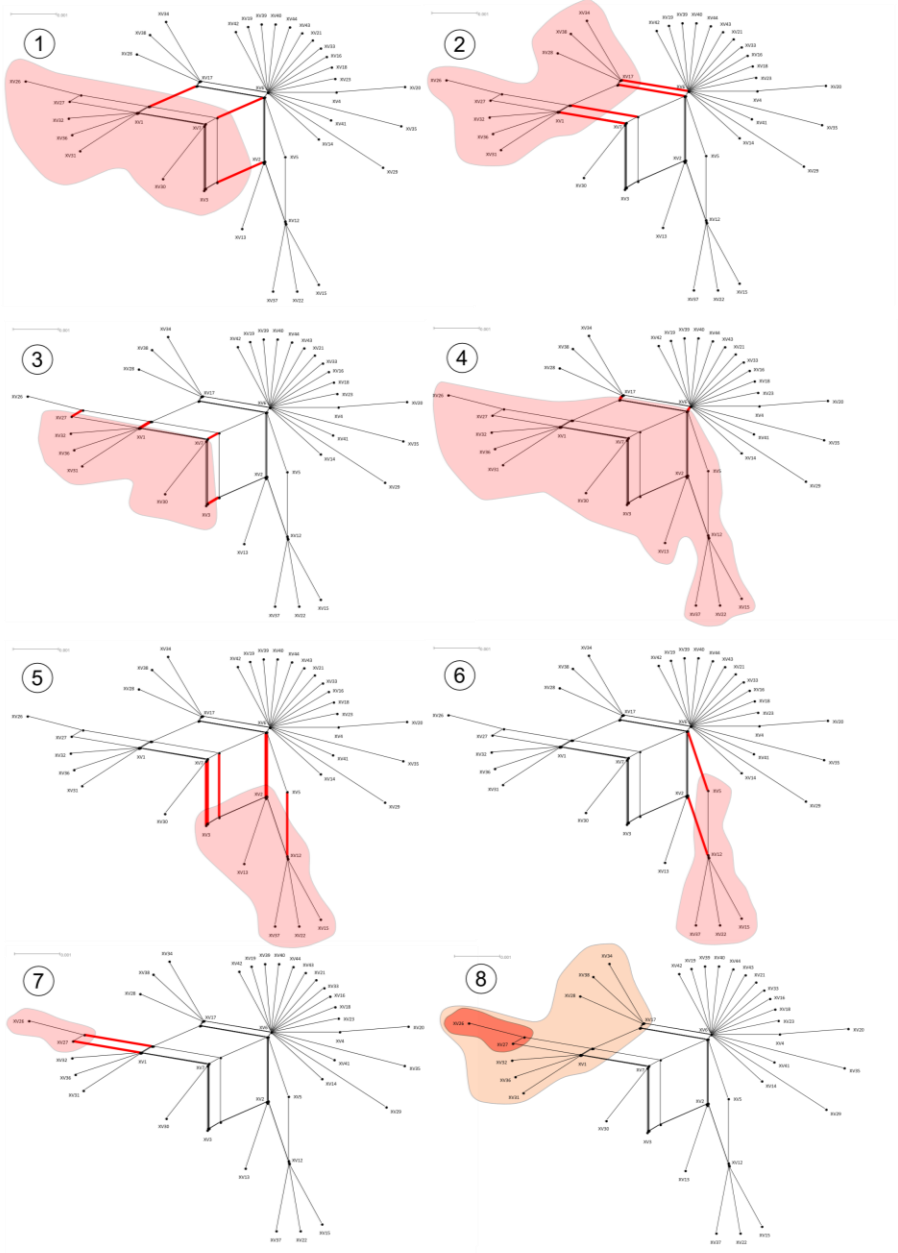

Figure VII.H.1. Step-by-step illustration of how to interpret a splits diagram using the *X. virginica* haplotypes found in this study. Splits are highlighted in red, with their corresponding haplotype groups shaded in red polygons. 1) [XV1+XV3+XV7+XV26+XV27+XV30+XV31+XV32+XV36] A major split noted by the relative length of the lines in the set; 2) [XV1+XV17+XV26+XV27+XV28+XV31+XV32+XV34+XV36+XV38] Another major split. Note that part of the group in this split is also a member of split #1; 3) [XV1+XV3+XV7+XV27+XV31+XV32+XV36] A smaller split, as shown by the shorter

Figure VII.H.1. (Cont.)

length of the split. Note that this group is a subset of split #1, but XV26 is excluded; 4) [XV1+XV2+XV3+XV5+XV7+XV12+XV13+XV15+XV22+XV26+XV27+XV30+XV31+XV32+XV36+XV37] A very small split that separates two very large groups; 5) [XV2+XV3+XV12+XV13+XV15+XV22+XV37] A large split; 6) [XV5+XV12+XV15+XV22+XV37] Another large split; 7) [XV26+XV27] A large split that separates a small group; 8) 8. These two groups were similarly recovered in the Bayesian analysis in Fig. VII.D.3. The orange group contains haplotypes exclusive to the eastern portion of *X. virginica*'s range, with the red subgroup exclusive to Florida. The two western clades (XV12+XV15+XV22+XV37) and (XV4+XV20) cannot be separated into exclusive groups by any of the splits in the network.

VIII. CONCLUSION

This work explores bee distributions from many perspectives, from how to detect declines on multiple geographic scales, through the community interactions that might govern local population dynamics, to the possible role that ecological interactions in the distant past had in forming species' ranges, all in an effort to provide much-needed background information on important taxa in a changing world. In Chapter II, I show that county-level records of state-wide bumble bee distributions can reveal the conservation status of bumble bee species. Even within Arkansas there is evidence that the nationally-declining species *Bombus pensylvanicus* (DeGeer, 1773) is on the decline state-wide. However, Chapter III shows that the genetic diversity of this declining species is equivalent to that of species shown to be stable, nationally and state-wide, suggesting that some recommended conservation-genetics tools might not be applicable at management-level scales. Chapter IV shows that, although diploid males can be an indicator of small population sizes and inbreeding in bumble bees, these can also be rather common in populations of stable species such as *B. bimaculatus* Cresson, 1863 and *B. impatiens* Cresson, 1863. This is the first report of diploid males in these species, and it highlights the need for additional data on the baseline expectations of diploid male occurrence before applying this as a monitoring tool. In Chapter V, I explore a number of ecological factors in a long-season bee community in Northwest Arkansas and find that on-site floral diversity is tied to local bumble bee abundance. Additionally, I find that, even though they are more specialized and overlap in their food choices, the long-glossa species *B. auricomus* (Robertson, 1903) and *B. pensylvanicus* rarely have the opportunity to compete because they have divergent active periods. This offers a novel explanation as to why bumble bee communities have more species than a strict competitive-exclusion hypothesis would predict. In Chapter VI, I revisit the subspecies of

Xylocopa virginica and find little support for retaining *X. v. krombeini* on morphological grounds, although *X. v. texana* seems sufficiently distinct from *X. v. virginica.* In Chapter VII, I find that *X. virginica* likely persisted through the last glacial maximum of the Pleistocene in multiple refugia, with evidence for one refugium on each side of the Mississippi River and additional refugia possible in the east. In contrast, *X. micans* seem to have dispersed from a single refugium. These results are consistent with the hypothesis that range size disparity between these two species is due to ecologically-mediated post-glacial colonization dynamics. However, *X. micans* seems to be undergoing a recent range expansion, occupying areas north of its historical range. The cause of this expansion is unknown, but bee distributions are clearly changing throughout the region. Some species, such as *X. micans,* appear to be expanding their ranges, while others such as *B. pensylvanicus* appear to have contracting ranges. Because pollinators are such a vital part of our ecosystems, further research into changing bee distributions is warranted.